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(57) Abstract

Material to be frozen is subjected to a cooling process which involves the efficient removal of latent heat of freezing. This can be achieved by subjecting the material being frozen to a greater rate of heat extraction when the latent heat is being given up than when the then solid material is being subsequently cooled further. Efficient removal of latent heat is also facilitated by inducing nucleation of the frozen liquid. Nucleation can be initiated acoustically and/or chemically. The invention, which has particular application in the frozen food industry and in the cryopreservation of biological material, allows shorter freezing times and/or improved quality or viability of the frozen product.

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COOLING PROCESS AND APPARATUS

This invention relates to a method of freezing a material and to apparatus for use in such a method.

The invention has particular application in a number of fields, as it can minimise the effects of undercooling during freezing in order to alleviate or avoid damage to the material being frozen. In particular, the invention may be used in:

(A) the frozen food industry;

(B) the cryopreservation of human embryos and embryos of other animals;

(C) the freezing of human organs for transplantation;

(D) the freezing of small or large volumes of cell suspensions, such as blood, bone marrow and microorganisms;

(E) the freezing of other biological material, particularly cellular (whether plant or animal) material; and

(F) the freezing of other material, particularly where freezing must take place in controlled conditions, for example, in freeze drying and/or in the production of highly regular crystalline solids.

It is necessary to freeze or solidify many materials in commercial and industrial processes. Freezing may be

part of a production process or be a means of enhancing the storage characteristics of the material. The storage of foodstuffs by freezing is a common method of maintaining their viability for long periods of time. Equally, in other technical fields, cryopreservation is recognised as the principal method of preserving biological material, particularly delicate and valuable material such as human or other animal embryos, until required for use. It is anticipated that there are further possibilities for the application of cryopreservation techniques to biological material: there is a major shortage of human tissues and organs for transplantation including corneas, pancreas, kidney, liver and heart.

Although the freezing of foodstuffs, the cryopreservation of biological material and the solidification of other materials may seem to be a disparate collection of industrial and commercial processes, in fact they tend to share a common major problem. During cooling of the "material" (which will be used as a generic term), liquid in the material (for example in medium surrounding cells in a biological sample) tends to supercool to a point below its freezing or solidification point before nucleation of the solid phase occurs. This is also known as undercooling. Supercooling or undercooling can cause damage to the material, and in the case for example of embryos can even prevent their survival, because of the following effect. (Although the discussion that follows relates to material comprising liquid water and the formation of solid ice, the same principles would apply to other liquid/solid systems.)

Conventionally, as an aqueous material is cooled at a steady rate, the temperature of the material will fall with the surrounding falling temperature until the nucleation point of the liquid is reached. Because of the tendency to supercool, this will be below the melting point. At the nucleation point, water in the material crystallises into ice, thereby liberating latent heat of fusion. The temperature of the material at this point rises from the nucleating point almost to the melting point. Once the latent heat of fusion has been lost by the material and/or its associated water, the temperature of the material again begins to fall. However, because the surrounding temperature has by this stage become cooler, there is a greater differential between the material temperature and the surrounding temperature, so the material cools much more quickly. This results in the relatively uncontrolled formation of ice crystals, whose large size can have a deleterious effect.

This leads to a real problem for the frozen food industry. A conventional technique employed by the food industry to freeze food is to use a blast or tunnel freezer where the food is cooled by cold gas. Inside the freezer these is a gradient of gas temperature, the temperature being warmest at the end at which the food is introduced and gradually becoming lower as the food passes through the freezer. Initially the sample cools in parallel with the gas temperature. However, after nucleation the food temperature rises to the latent heat plateau. Here, the rate of loss of heat from the food to the environment is proportional to the temperature

difference which increases while the latent heat is being given up. The food is therefore buffered at this exotherm until the latent heat of fusion has been dissipated, at which time the temperature of the sample will then rapidly equilibrate to the environment temperature, resulting in a sharp drop in temperature.

In the frozen food industry, products such as some soft fruits (eg. peaches, plums, raspberries) and seafoods (eg. lobster, crab, prawn, finfish) are often of poor quality when thawed. With other soft fruits (eg. strawberries, kiwi fruit, mango), various vegetables (such as new potatoes and asparagus) and some dairy products (for example single cream) the problem is more extreme and these products are not frozen on a commercial basis. A major component of such freeze-thaw injury is the loss of texture due to mechanical damage caused by uncontrolled nucleation of ice crystals and their subsequent growth associated with prolonged periods at the latent heat plateau.

The quality of products which are consumed in the frozen state such as ice cream, sorbets and ices are related to the size and distribution of ice crystals, formation of which is often difficult to control. Furthermore, in conventional freezing methods, water in the sample nucleates on the outside and ice propagates towards the centre. The evolution of latent heat at the periphery of the sample results in the core being thermally buffered and "shell" freezing occurs.

With the cryopreservation of sensitive biological cellular material, cellular material, there is an

additional harmful effect resulting from supercooling or undercooling. As ice forms in the medium the concentration of any solutes in the remaining liquid increases. By osmotic pressure, the cells will thus dehydrate, as a result of water moving to the more concentrated medium. If the cells have insufficient time to dehydrate, then intracellular ice may form, which is generally lethal to the cell.

In order to minimise the potential problems caused by supercooling, EP-A-0246824 teaches that a range of solid materials can be used to cause water in an aqueous medium to be nucleated at, or close to, the freezing point of the medium. However, even with this considerable improvement over prior methods, care still needs to be taken in otherwise conventional cooling methods that damage does not occur during the relatively rapid cooling period after the temperature plateau during which at least some of the latent heat of fusion of the medium is being lost.

The above discussion has centred on material comprising (and in particular containing a significant amount of) water. Water has a strong tendency to cool below its freezing point (the supercooling or undercooling effect) which introduces complications in cooling of biological tissues which have many membrane bound compartments which limit the propagation of ice. A variety of methods have been described to initiate ice nucleation. A number of inorganic compounds, silver iodide being a common example, and organic compounds (see EP-A-0246824, discussed above) and "ice nucleating" bacteria (members of the genera

Xanthomonas, Pseudomonas, and Erwinia) have been demonstrated to have a crystal lattice structure which are effective nucleators of ice in supercooled water. Whilst these compounds have applications, for example in the seeding of rain clouds, biological cryopreservation and snow formation respectively, they cannot be readily applied to foodstuffs due to toxicity, legislation or problems of application.

The problems of uncontrolled nucleation have been seen effectively to prevent the commercial freezing of certain foodstuffs, as discussed above. Although similar (or worse) problems have arisen in the somewhat more specialist field of cryopreserving biological samples, some attempts have been made to initiate nucleation in a relatively controlled manner, in addition to the seeding process described in EP-A-0246824. For example, ice nucleation has in the past been initiated by either (a) mechanical shaking, (b) thermoelectric shock, (c) thermal shock or (d) direct addition of ice crystals.

Mechanical shaking is an inefficient cumbersome process that may damage the sample. Thermoelectric shock can be delivered by supplying a current across the sample in the case of a solid or container enclosing a liquid sample. The technique uses the reverse of the Peltier thermocouple effect. Thermal shock may be achieved by contact of the sample with a much colder surface or the insertion of a precooled surface such as a metal wire or glass rod. Perhaps the least inelegant of the present processes is the direct addition of ice crystals to a liquid sample or the surface of a solid.

These last three invasive processes are unsuitable for foodstuffs. There is therefore a need for an improved non-invasive method of avoiding the serious consequences of supercooling and subsequent nucleation.

The present invention addresses the problems discussed above and provides a surprisingly simple and elegant solution, which can be put into practice in a variety of relatively straightforward ways.

At its broadest, the invention provides, in a first aspect, a method of freezing material comprising a liquid, the method comprising extracting heat from the material and varying the rate of heat extraction to compensate at least in part for latent heat being lost during freezing.

More particularly, according to a second aspect of the present invention, there is provided a method of freezing material comprising a liquid, the method comprising extracting heat from the material at a first rate while latent heat of fusion of the material is being lost from the material and the temperature of the material is not substantially falling and subsequently extracting heat from the material at a second rate when the temperature of the material falls, the first rate of heat extraction being greater than the second rate of heat extraction.

The invention therefore seeks to minimise or at least reduce the amount of time the sample spends at the temperature "plateau" during which the latent heat of fusion is being lost. In relation to the freezing of

biological samples, there is evidence (Parkinson and Whitfield, Theriogenology 27 (5) 781-797, (1987)) that the survival of cryopreserved bull spermatozoa is inversely related to the time at the latent heat plateau; however, Parkinson and Whitfield appear to advocate a lower cooling rate between 5° and -15°C than between -15°C and -25°C. The problem is however not restricted to the viability of living systems: for foodstuffs in particular, an excessively long time at the latent heat plateau leads to damage mediated mechanically by the effects of ice crystals and chemically by unusual osmotic effects, for example, in the semi-frozen state. It has been observed that longer periods of time at the latent heat plateau lead to the formation of longer ice crystals and to a degeneration in quality of the subsequently thawed product.

By means of the heat extraction regimen of the method of the present invention, the cooling rate can be controlled so that the material being frozen suffers few or no deleterious effects. In particular, as at least some of the latent heat of fusion is being given up by the material, the heat extraction rate is greater. However, the temperature of the material will not substantially decrease during the period when significant quantities of the latent heat of fusion being given up by the material. After at least some of the latent heat has been given up, the lesser rate of heat extraction is necessary so as to prevent too great a range of temperature drop. The first rate of heat extraction may therefore take place when the temperature is increasing or constant or the rate of

temperature drop of the material is not substantial (for example, less than 1°C/min or even 0.1°C/min), and the second rate may be applied when the rate of temperature drop is at least 0.1°C/min or even 1°C/min.

The invention may also permit a shorter dwell time in a freezing apparatus, before transfer of the material being frozen to a cold storage environment, and this may be of significant advantage.

It should be noted that the use of the term "rate" as applied to heat extraction does not imply that either the first or second rate of heat extraction is constant. Either or both rate may vary, and in some instances a variable heat extraction rate may be preferred, to achieve non-linear and/or interrupted cooling. An "interrupted cooling" profile includes a profile having an initial rate of cooling, followed by an isothermal hold, which in turn is followed by a subsequent cooling rate (which may or may not be the same as the initial cooling rate). Non-linear and interrupted cooling profiles have biological and non-biological application. Overall, in this invention the second heat extraction rate must be less than the first.

It should also be noted that the term "first", as applied to heat extraction rate, does not preclude the use of a different heat extraction rate prior to the latent heat temperature plateau being reached.

It will be understood that the word "frozen", as used in this specification when applied to complex mixtures

of solvent(s) and solute(s), such as biological material and/or foodstuffs, does not necessarily imply that all matter in the material is in the solid state. For example, to take the case of a frozen foodstuff such as strawberries at -25°C, about 10% of the fruit will be liquid at that temperature, yet the strawberries would in ordinary parlance be referred to as "frozen": it is in this sense that the word "frozen" is used, and cognate terms should be construed accordingly.

The second rate of heat extraction will determine the rate of cooling of the solidifying or solid material. The rate of cooling selected should be such as not to damage the material, for example by enabling significant ice crystals to form in aqueous systems.

The second rate of heat extraction will vary widely, depending on the nature of the material. For mammalian embryos, for example, the second heat extraction rate should be such that the cooling rate does not exceed 0.5°C/min and should preferably be about 0.3°C/min at least in the range of -5° to -30°C. However, for reasons of expediency, within these limitations cooling should be as rapid as possible. Although these criteria apply to mammalian embryos, other materials may have their own criteria; for example, samples containing hybridomas, lymphocytes, tissue culture cells (eg mammalian) and various microorganisms may be cooled at a greater rate, for example from 0.5°C/min to 1.5°C/min, such as about 1°C/min. For other material, for example oyster embryos the cooling rate may be about 5°C/min, and for red blood cells, the rate may be

several thousand °C/min, for example up to about 3000°C/min.

In this invention, the first rate of heat extraction is applied while latent heat of fusion of the material is being lost. This should not be taken to mean that all of the latent heat of fusion has to be lost during the application of the first rate of heat extraction. In any aqueous sample, for example, latent heat will be liberated from the temperature of nucleation down to the eutectic temperature or the glass transition. However the majority (for example at least 70% or 80% or even at least 90%) is generally liberated at the freezing point and a few (for example 5 or 10) degrees celcius below. The first rate of heat extraction is for preference applied while a majority (for example at least 80% or even at least 90%) of the water is converted into ice, which is to say while a majority (for example at least 80% or even at least 90%) of the total latent heat of fusion of the material is being lost.

From phase diagrams of simple solutes such as sodium chloride, the amount of unfrozen water in the system can be seen to decline exponentially with temperature. At any sub-zero temperature, the proportion of unfrozen water is directly related to the osmolarity of the unfrozen solution. For solutions of interest to the food industry (for example 0.5 and 0.25M sodium chloride solutions and their equivalents) 80% of the ice will have formed by -10°C.

The invention can therefore be seen to embody the notion of efficient removal of latent heat during freezing or, in preferred embodiments, during the conversion of, say, 80% of water into ice. In those systems where phase diagrams cannot be derived, then the efficient removal of latent heat from the melting point (ie the latent heat plateau) to 5°C or 10°C below the melting point. Although efficiency is to some extent a relative concept, in certain embodiments of the present invention latent heat removal (for example to the extent referred to above) may be considered efficient if it is achieved in 50% or less than 50% of the time observed when following conventional blast freezing techniques at -30°C.

The method is particularly applicable to the freezing and cryopreservation of biological samples, which thereby constitute preferred examples of material which can be frozen by means of the invention. The term "biological sample" includes cells (both eukaryotic and prokaryotic), organs and tissues composed of cells, embryos, viruses, all of which can be natural or modified genetically or otherwise, and biologically active molecules such as nucleic acids, proteins, glycoproteins, lipids and lipoproteins. The liquid present in or constituting the material will generally be water, but the invention is not limited to aqueous materials.

The invention may be used in the cryopreservation of animal cells, particularly gametes or fertilised eggs/embryos. However, other animal cells and plant cells can advantageously be frozen by means of this invention.

Another significant application for the invention is in the frozen food industry, where it may be important for reasons of preserving taste and/or texture or otherwise to freeze food quickly and efficiently and without causing excessive damage to the biological or other material which constitutes the food. For example, soft fruit when frozen by conventional means loses much of its taste and/or texture. The material is thus preferably a foodstuff, such as vegetables, bread and other bakery products, meats, fish, sea food (eg. lobster, crab, prawns, finfish) or fruit, in particular soft fruit such as peaches, plums, raspberries, strawberries, kiwi fruit and mango. Non-aqueous systems and emulsions, such as chocolate (whether plain, milk or white), ice cream, cream and mayonnaise, may also be frozen by means of this invention, as may reconstituted food products.

The invention also has application to non-biological material which needs to be frozen in a controlled fashion. This may be necessary or desirable for certain foodstuffs and/or other material in which the rate and nature of crystal formation is important. Sorbets and ices may fall into this category.

The invention can also be applied to the cryopreservation of organs for transplantation and large volumes of cell suspensions such as blood, bone marrow and microorganisms.

The volume of the sample to be frozen is not particularly critical, but when freezing or cryopreserving gametes or fertilised egg/embryos in the

biological sciences, the sample volume will generally be less than 1ml, typically less than 0.5ml and may even be less than 0.2ml. Volumes of 0.5ml and 0.25ml are common. For the frozen food industry, the volumes to be dealt with will of course be much larger, often several dm^3 or even m^3.

Particularly in the case of cryopreserving biological samples for scientific, clinical or commercial use, the material to be frozen may be in a container or on a carrier. Suitable containers include ampoules, tubes, straws and bags (particularly thin-sectioned bags, which may be held between two heat conductive (eg metal) plates). Appropriate polymers include plastics materials such as polypropylene or polyvinyl chloride. Containers which are small in at least one dimension are preferred, as temperature gradients may then be ignored across the small dimension or dimensions. Tubes, straws and thin-sectioned bags are particularly preferred for this reason.

In a further important aspect, the invention involves the use of acoustics, particularly acoustics of the type generally known as high frequency sound or ultrasound. The application of acoustics/ultrasound to improve the crystalline structure of metal castings is known as dynamic nucleation. Whilst acoustics/ultrasound may induce nucleation in supercooled metals, the predominant benefit is grain refinement. Irradiation with acoustics also improves heat transfer at the boundary layer. Nucleation of ice formation by acoustics has received scant attention in the past. For example, Hobbs ("Ice Physics", Clarendon

Press, Oxford, 1874) which is regarded as a standard work in the area, does not metnion the potential of acoustics in ice formation. Two Russian patent documents, with commercially impracticable teachings are however known.

In SU-A-0618098 food products were stated to be frozen more rapidly and their quality improved by placing in a coolant and simultaneously exposing to ultrasound at 18-66 kHz and 16-40 W. The treatment was stated to increase heat exchange at the boundary layer and caused ordered formation of finely-crystalline ice. The document does not disclose ice nucleation, but, by reference to and inference from the metallurgy industry, grain refinement is probably the result of ultrasonication.

SU-A-0395060 teaches a similar process where the freezing process time was reduced from 5 min 10 sec to 3 min 5 sec, clearly a manifestation of improved heat transfer. Ultrasound was also stated to exert a beneficial effect on crystalisation processes, but again nucleation by the ultrasound was not stated. Both these processes are, however, commercially unacceptable as disclosed for a number of reasons.

First, it has been found that when the process was repeated with strawberries or strawberry slices (4.5mm) the thawed product was of unacceptable quality. There was no detectable improvement in the quality of the fruit compared with material frozen in a conventional (-30°C) blast freezer without the use of ultrasound.
Secondly, the processes described require immersion of

the food in a bath of either ethylene glycol (-22°C) or freon 12 (-29.8°C). The possibility of contamination of the food with either of these substances would be an unwelcome risk under commercial circumstances, and the cost of these chemicals may in practice prove prohibitive.

Thirdly, the power that is used (2 to 3 w/cm^2) is very high: this will not only have a severe warming effect on the food, it may also induce cellular damage to material being frozen.

After nucleation of ice within a food the latent heat of fusion should be removed as quickly as possible to minimise the effect of supercooling. It is known in the food freezing industry that to achieve this the samples may be immersed into cryogens, such as liquid nitrogen (-196°C), liquid CO_2 or freons, but this has several associated problems.

First, with large biological samples (such as above 5mm diameter) "shell" freezing will occur resulting in fracture and cracking of the sample.

Secondly, in some fruits, such as strawberries, a secondary type of tissue damage occurs if the fruit is cooled below -100°C. It is extremely difficult to conduct a liquid nitrogen immersion process without causing damage by exceeding the minimum storage temperature.

Thirdly, the immersion of samples into liquid nitrogen is a costly process and therefore uneconomic and likely

to be unsustainable in the frozen food industry.

The teachings of SU-A-0618098 and SU-A-0395060 may be unworkable on a practical basis if directly applied to freezing liquid-containing material such as biological material and/or foodstuffs, and it appears that the frozen food industry has largely ignored the possibility of using acoustics in freezing processes.

It has now been discovered that the use of sound, particularly high frequency sound, is highly benefical when used in conjunction with or even independently of a heat extraction method in accordance with the first aspect of the invention. Preferably, therefore, the material being frozen is subjected to sound waves, which may be high frequency sound waves.

The high frequency sound waves are preferably ultrasound waves, generally at a frequency of at least 16 kHz, for example from 18-80 kHz. The frequency at which acoustics is preferably applied ranges from 20 kHz to 50 kHz. Typically the applied frequency is from 20 kHz to 30 kHz; the optimal range for at least some applicatons appears to be from 22.5 kHz to 25 kHz.

Supercooled material may be subjected to the sound waves for from 0.1 to 1.0 seconds. Alternatively, the material may be pulsed or otherwise supplied with acoustics throughout the freezing process. It is preferable for the acoustics to be applied as one or more pulses. The pulse duration should on average preferably be from 5% to 20% of the total time of pulse-plus-interval; preferably the pulse lenth is from

0.5 to 5 seconds, with about 2 seconds being optimal. Pulses of about 2 seconds in 20 seconds have been found to be particularly effective. The power and/or frequency may be varied (either discreetly or continuously) during application. More than one frequency may be used at the same time. It may be particularly appropriate to apply acoustics when certain material being frozen is in the liquid phase; this may apply in particular to ice cream.

As far as the power at which the acoustics is applied, there is clearly a conflict in requirements. On the one hand the power should be high enough for the acoustics to be effective, and on the other hand the power should not be so high as to cause unacceptable heating of the material being frozen (as the energy applied will be dissipated as heat). Power applied between 0.05 and 1.9 or 2.0 W/cm^2 was found to be acceptable, with a range of 0.1 to 1.5 W/cm^2 being preferred and about 0.2 to 1 W/cm^2 being optimum.

This non-invasive technique of inducing ice nucleation thus at least mitigates, or overcomes, problems associated with prior art techniques.

The sound waves may be generated by sound wave generators known in the art, such as ultrasonic baths, piezoelectric transmitters and suitable transducers. Thus the material may be in contact with the sound wave generator, for example inside a container such as a mould in contact with a piezoelectric transmitter, or on a conveyor belt in contact with a suitable transducer. In this latter embodiment the material may

thus be moved within an environment having a temperature gradient, such as a conventional blast or tunnel freezer.

Four preferred methods of inducing ice nucleation using high frequency sound waves are as follows.

1. The sample is immersed in an ultrasonic bath which is preferably maintained at, or about, the freezing temperature of the material (eg. -20°C). Thus the sound wave generator serves to both provide the high frequency sound waves and also to cool the material. The material will generally be immersed in a liquid, preferably an aqueous liquid, such as water. However, the material, if desired, may be contained or enclosed in a mould which is particularly suitable for the freezing of ices.

2. The material may be placed in a container, such as a mould, which is cooled in a freezing bath. A piezoelectric transmitter is placed in contact with, or built into, the mould to deliver the high frequency sound waves. This method is particularly suitable for frozen sorbets, ices and ice creams.

3. The material may be placed on top of a conveyor belt which is in contact with, or interrupted by, one or more transducers. This method is particularly suitable for thin layers of material, such as slices of foodstuffs such as soft fruits. The contact between the material and conveyor belt ensures that the sound waves are transmitted efficiently to the whole of the material. Cooling of the material can be achieved by

passing the conveyor belt through, for example, a conventional blast freezer. It is preferred that a short zone of acoustic transducers is placed at a particular point along the conveyor belt to achieve maximum nucleation in the material.

4. For larger materials and those of non-planar geometry, such as spheres and cylinders, to achieve more than a point contact with an ultrasonic source, it is preferable to immerse the sample either fully or partially in a liquid in a container. The high frequency sound waves can then be applied via transducers, but the material will be immersed in the liquid for only a short period (for example less than one second). The temperature of the container is preferably maintained so as to keep the material at its freezing temperature, for example about -5°C. The liquid in the container is preferably kept below its freezing point by the addition of non-toxic chemicals, for example food grade chemicals. This has the advantage that the material may be simultaneously coated with the food grade chemical. Preferred food grade chemicals include sugars and glycerol, for example to freeze the material and add a glaze. This embodiment may be combined with a continuous process such as the material being carried along a conveyor belt as discussed above. For example, the conveyor belt may dip into an ultrasonic bath, suitably for a short period such as less than one second, when it is subjected to ultrasound.

The material is preferably precooled before subjection to the high frequency sound waves to induce ice

nucleation. Suitably the material will be cooled so that it is at the same temperature, namely of thermal equilibrium, as the environment. This is since if a large temperature difference exists between the material and its environment then a temperature gradient will be established across the material and nucleation will occur on the outside and the ice front will propagate towards the centre, resulting in unwanted "shell" freezing. Thus, if the whole of the material is precooled to the temperature of the environment, and in particular such that the inside of the material is at the same temperature as the environment, then on subjection to the high frequency sound waves ice nucleation may be induced on the inside and preferably at the centre, of the material. Usually the material will be thermally equilibrated with the environment below its freezing point.

The application of acoustics, as preferred for the present invention, as described above, itself forms an independent aspect of the invention. It has been found that if the immersion techniques suggested in the Russian patent documents described above is avoided, it is possible for acoustics to be beneficial and commercially feasible. According to a further aspect of the invention, there is provided a method of freezing material comprising a liquid, the method comprising abstracting heat from the material and applying sound waves to the material by means of a non-liquid contact with the material. Generally, there will in this aspect of the invention be solid or mechanical contact between a source of high frequency sound waves and the material to be frozen, but

gas-mediated contact may be adequate. The contact may for example be achieved by the use of a source of high frequency sound waves in the form of a probe, such as the BRANSON LUCAS-DAWE probe, in direct contact with the material. Alternatively or additionally, the material could rest on a solid surface, to which was mechanically connected, directly or indirectly, a source of high frequency sound. It will be appreciated that a layer of suitable material may be interposed between the material to be frozen and the solid surface, for example to prevent contamination and/or undesirable sticking, but this is not to be regarded as detracting from the mechanical connection, which is just rendered somewhat more indirect. Further, it is to be understood that uniform contact between the material and the surface is not necessary: it is only necessary for there to be sufficient contact for the sound waves to be transmitted effectively.

A fluid-filled (preferably liquid-filled) layer may be interposed in the sound path between the source of high frequency sound and the material to be frozen. This is not to say that liquid is in contact with the material to be frozen; on the contrary, the fluid layer simple aids transmission and/or distribution of the high frequency sound waves into the material. the fluid may be any organic solvent, but is preferably freon, glycol, ethanol or a food-compatible solvent such as sold under the trade mark ISOPAR. The ISOPAR K product may be the most preferred.

It is to be understood that the "non-liquid contact" of the material to be frozen does not necessarily imply

complete dryness. For example, if cut fruit is being frozen, a small amount of liquid may be released from the fruit itself. This is however to be contrasted with immersion within a sound-transmitting liquid, which is not within this aspect of the invention.

It has also been discovered that if the relatively high power levels taught in the Russian patent documents referred to above are avoided then, contary to expectations the results are better; further, a lower power level can be delivered by a more economical piece of equipment. According to a further aspect of the invention, there is therefore provided a method of freezing material comprising a liquid, the method comprising abstracting heat from the material and applying sound waves to the material at a power level of less than 2 W/cm^2. Preferred features of this aspect of the invention are as described above.

Further, intermittent application of acoustics may provide the basis for improved performance over the disclosure of the Russian patent documents.

Correspondingly, the invention relates in further aspects to an apparatus for freezing material comprising a liquid, the apparatus comprising means for abstracting heat from the liquid and means for applying sound waves to the material, wherein (a) the sound waves are applied to the material by means of a non-liquid contact with the material and/or (b) the means for applying sound waves to the material is adapted to deliver the sound waves at a power level of less than 2 W/cm^2 and/or (c) the means for applying

sound waves to the material is adapted to deliver the sound waves intermittently. Preferred features are as described above.

Methods in accordance with the invention work well in conjunction with the use of other means for inducing ice to nucleate, such as by using chemical (for example crystalline) ice nucleators, such as is disclosed in EP-A-0246824. Such nucleators can be used to determine reasonably accurately when ice nucleates. The nucleator may be coated on one or more walls of a container for the material and/or on a carrier for the material. As is disclosed in EP-A-0246824, cholesterol is a preferred nucleator.

Heat extraction may be achieved by any convenient way. In principle, it is possible for heat to be extracted by an endothermic reaction taking place in the material. However, it will usually be more convenient to provide a temperature gradient between the material and at least part of the surrounding environment, which should be cooler than the material. This embodiment of the invention takes advantage of Newton's law of cooling, which states that the heat loss will, for small temperature differences be proportional to the temperature difference between the material and the surroundings.

Heat extraction can therefore most easily be achieved in many applications of the present invention by placing the material in a cold environment. It therefore follows that, to achieve first and second heat extraction rates where the first heat extraction

rate is greater than and followed by the second, the sample can be moved from a cold environment to a less cold environment, for example by means of a conveyor system. In practice in some applications, it may be easier to change the environment temperature rather than to move the sample, in which case the environment temperature is increased at the interface between the first and second rates.

Suitable environment temperatures for the first and second heat extraction rates will be apparent to those skilled in the art. For preference, the environment temperature for the first heat extraction rate will be at least 15°C, and preferably at least 25°C lower than the environment temperature for the second heat extraction rate. When the material to be frozen comprises water, for example in the case of biological material such as organs or, particularly, foodstuffs, the environment temperature for the first heat extraction rate can be for example less than -50°C, or even -80°C or -100°C; the environment temperature for the second heat extraction rate may be -20°C to -30°C. For foodstuffs, the environment temperature for the second heat extraction rate may be the final desired storage temperature. For biological material that is to be cryopreserved, it may be desired to reduce the environment temperature further, for example after the second heat extraction rate.

The preferred minimum environment temperature for the first heat extraction rate may in part be determined by tolerance of the material being frozen to temperature gradients. For fruit at least, and possibly for other

foodstuffs and biological material, placing material to be frozen which has equilibrated with room temperature in an environment temperature for the first heat extraction rate of -100°C or less appears to cause too large a temperature gradient to be acceptable in some circumstances. Strawberries, for example, suffer injury under such conditions, possibly caused by the non-uniform formation of glasses and eutectics.

As an alternative to altering the environment temperature, different rates of heat extraction may be achieved by altering the efficiency with which the environment extracts heat from the material: cold air or other gas may be passed over the material at different rates for this purpose. A higher gas velocity will achieve a higher heat extraction rate, as can be found with everyday experience of wind chill factors.

It will be appreciated that the present invention can be put into effect by making adjustments and modifications to enable the appropriate heat extraction protocol to be carried out. As discussed above, this may be achieved by an appropriate protocol for changing the environment temperature. Such protocols can readily be established for various foodstuffs and other biological material by taking into consideration the relevant parameters for each material, for example including:

a) Size;
b) Geometry;
c) Water content;

d) Freezing point (to a first approximation this is dependent on solute concentration within the foodstuff or other material);

e) Thermophysical values of the material of the material, both before freezing and in the frozen state; and

f) Container dimensions and other details.

Because these parameters differ from material to material a computer can readily be used to derive optimum protocols.

The temperature history in a sample being cooled in a controlled rate freezer (such as the KRYO 10 series Chamber Model 10-16 by Planar Biomed, Sunbury-on Thames, England) can be calculated by solving numerically the Fourier heat conduction equation in the sample with convective or other boundary conditions as appropriate. (The expression KRYO 10 is a trade mark.) In general, the calculation method must allow for the cooling of an aqueous solution or other material where compositional as well as phase changes occur during freezing. This requires the appropriate molarity-freezing point depression data to be available, to provide the relationship between ice formation and melting temperature. Supercooling of the sample may also be suitably accounted for. In the case of thin slices the temperature gradients across the sample can be assumed negligible and consequently the conduction equation reduces to a simple unsteady heat balance between the time rate of change of enthalpy of the sample and the heat transfer rate across its boundaries. The validity of this simplified

calculation has been compared against experimentally derived data. The calculation method has been employed to predict methods to reduce the latent heat plateau within plum slices by manipulation of the environment temperature.

However for calculating the temperature history in samples of finite thickness, where conduction within the sample is important, it is necessary therefore to solve the full equation. Solving the full unsteady equation with three space dimensions is computationally very time consuming. However, in many cases the temperature gradients in one direction are much greater than in the other two and in these systems a reasonable prediction for the temperature history can be obtained from a one-dimensional model. This model could be developed for 1-d Cartesian, 1-d spherical or 1-d cylindrical geometry.

In its broadest apparatus aspect, the invention provides an apparatus for freezing material comprising a liquid, the apparatus comprising means for extracting heat from the material and control means for varying the rate of heat extraction to compensate at least in part for latent heat being lost during freezing.

According to a further aspect of the invention, there is provided an apparatus for freezing a material comprising a liquid, the apparatus comprising means for extracting heat from the material at a first rate while latent heat of fusion of the material is being lost from the material and the temperature of the material is not substantially falling and means for subsequently

extracting heat from the material at a second rate when the temperature of the material falls, the first rate of heat extraction being greater than the second rate of heat extraction.

As discussed above, the apparatus will preferably comprise a (preferably high frequency) sound generator. The medium through which the sound is conducted from the generator to the material may be gaseous, for example air, or solid.

Each heat extraction means can in general comprise a refrigerated element, which may actively be cooled by expansion of a gas. Conventional diffusion or compression/expansion refrigeration equipment may be used in this embodiment. However, this is not the only form of heat extraction means that can be used. For example, a cold liquid or solid which is dissipated as heat is extracted from the material can be used. An example of a cold liquid that can be used in this way is liquid nitrogen, which will be the material of choice for at least one of the heat extraction means for cryopreservating biological material, as biological material is conveniently stored at the temperature of liquid nitrogen. A cold solid which is similarly dissipated is solid carbon dioxide (dry ice), although the cooling effect of solid carbon dioxide will be less than the cooling effect of liquid nitrogen, because the sublimation point of the former is higher than the boiling point of the latter. A third possibility for a heat extraction means is to use a heat sink which warms up to equilibrium with the material to be frozen, or as nearly as any intervening (for example insulating)

material allows in the time available. The heat sink can therefore be a block of relatively cold material, especially a material with high heat conductivity, for example a metal. To counter any adverse problems of condensation, the metal will preferably be non-corrosive, for example by being made of brass or stainless steel. However, any metal can be used if appropriately protected, if necessary.

Suitable insulating material may be polystyrene (expanded or unexpanded) or another plastics polymer such as polytetrafluoroethylene or acetal but it will be appretiated that any material with suitable properties can be used.

An apparatus in accordance with the invention can comprise a single heat extraction means, such as one of those discussed above, and control means to control the single heat extraction means to extract heat at the first and second rates. For example, a so called "active" system in accordance with this embodiment of the invention could comprise a refrigerated element, control means and temperature feedback means. The control means could comprise a computer, microprocessor or other electronic means. The temperature feedback means would continuously or continually monitor the temperature of the material to be frozen and relay this information to the control means, which could then cause the refrigerated element to extract heat at the appropriate rate. Such an active system as this gives considerable flexibility for a wide variety of material to be frozen (particularly foodstuffs), but may involve relatively high expense for small amounts of material.

A similar but simpler embodiment could comprise a refrigerated element which is operable at two rates of heat extraction. The element may be arranged to operate first at a higher heat extraction rate, and then a timer may cause the element to switch to operation at a low heat extraction rate. Such an embodiment can be used when the characteristics of the sample, or at least the environment surrounding the sample, are known, but this may be acceptable in many circumstances, especially when various samples are small compared to the apparatus of the invention, so that any individual variation in characteristics will be relatively small.

Other preferred embodiments of "active systems" are as follows:

1) Batch systems. Mechanical freezers are generally cooled by the Joule-Thompson effect and operate at temperatures down to -80°C; a minimum of -135°C is possible. Material is placed into a closed chamber and left until it has reached the desired temperature and then removed for storage. The air in the chamber may be unstirred or fan driven to achieve forced convection. Additionally, the material to be frozen may be placed statically on shelves or rotated within the freezer.

The desired thermal profile may be obtained in such a closed system by direct control of the compressor temperature by electrical or mechanical means. In some cases this may be practically difficult as the response

time of such a control system may be too slow to generate the desired profile. However, as the minimum operating temperature will be required at the beginning of the process the control of temperature may be achieved by maintaining a constant compressor temperature whilst varying the heat input into the system from an independent heater which is programmed electrically or mechanically to generate the desired profile. In addition, a combination of direct control of compressor output together with an external heater may be employed. The control of temperature may be preprogrammed or alternatively may be actively controlled from temperature sensors placed either in the gas or in the samples to be frozen.

2) Continuous Systems. The material flows through the freezer on a horizontal conveyor belt or spiral system. Following a retention time within the freezer, the material emerges at a temperature suitable for storage. Gas circulation is usually fan driven; in some cases the cold gas is forced upwards through a perforated conveyor belt so that the samples are suspended as in a fluidised bed. The temperature at the point of entry is invariably warmer than at the point of exit. Cooling may be by mechanical means or alternatively by vapour from a cold liquid such as liquid nitrogen; in this case the minimum operating temperature achievable (>-160°C) is lower than in mechanical systems.

The desired thermal profile is to be achieved by the manipulation of the temperature distribution of the gas through the system. In contrast to the conventional

mode of operation the system will be at its minimum temperature at the point of entry of the food and will become warmer towards the point of exit. The temperature gradient within the continuous system may be determined in several ways, including a system of baffles to ensure the recirculation or removal of cold gas, the introduction of warm gas or the positioning of heaters. The velocity of gas flow will also modify the heat transfer and will be selected to be at its maximum at the point of entry, at later stages the flow may either be constant or reduced. In addition, the temperature experienced by the sample may also be modified by control of the speed of the conveyor belt. By employing a series of conveyor belts running at different speeds, the retention times within different areas of the freezer may also be manipulated. A combination of several of these processes may also be appropriate. The control of temperature may be preprogrammed or alternatively be actively controlled from temperature sensors placed either in the gas or in the samples to be frozen.

3) Immersion in low temperature baths. This is a process generally applied to ices, sorbets etc which are poured as liquids into moulds which are then semi-immersed in a stirred low temperature bath, typically at temperatures of -30°C. Such low temperature baths are usually cooled by contact with a heat exchanger cooled by the Joule-Thompson effect. Following freezing the sample is removed from the mould and placed into storage. The direct immersion of non-moulded foods into liquid cryogens is generally not considered good practice. However, immersion into

liquid CO_2, which is considered to be non-toxic and which evaporates at conventional storage temperatures, may be safely employed for a variety of foodstuffs.

The temperature profile achieved by immersion could be modified by several potential methods. A series of baths, maintained at different sub-zero temperatures could be employed, with the samples being immersed in sequence through the various baths. Alternatively, the thermal gradient along a single bath may be manipulated to achieve the desired profile, the rate of passage through such a gradient bath could also be modified in a linear or non-linear manner to achieve the desired profile. Again the control of temperature may be pre-programmed or alternatively may be actively controlled from temperature sensors placed either in the fluid or in the samples to be frozen.

In a quite different embodiment of the invention, apparatus in accordance with the invention can have separate heat extraction means for providing the first and second heat extraction rates, respectively.

What may be a preferred arrangement is again to have first and second extraction means, but to have the heat extraction means so arranged that together they provide the first heat extraction rate, whereas only one of them (for example the first heat extraction means) provides the second heat extraction rate. This arrangement gives rise to a particularly effective arrangement, particularly for the cryopreservation of biological material. The first heat extraction means may be a bath of liquid nitrogen or an environment of

cold nitrogen gas (eg above a bath of liquid nitrogen), which may be below -100°C. Biological or other material to be frozen can be contained in a Dewar flask also containing a cold (eg gaseous nitrogen) environment; the material can be appropriately insulated to provide an acceptable second rate of heat extraction. The cold gaseous nitrogen environment may for preference be provided in a specialised vessel known as a "dry shipper" with which those skilled in the art will be familiar or, less preferably, above a liquid nitrogen bath. As a further possibility, commercial deep freezes may provide an adequate cold environment; they are frequently capable of achieving and maintaining temperatures of from -80°C to -135°C. More generally, mechanical commercial freezers can have operating temperatures from -20 to -140°C, and liquid/gas freezers based on cryogenic gases can operate below these temperatures down to, or at least towards, absolute zero.

To augment the heat extracting effect of the nitrogen or other primary coolant to a degree sufficient to provide the greater first rate of heat extraction, a second heat extraction means may be provided during the time at which the first rate of heat extraction occurs. Appropriately, the second heat extraction means may be a heat sink, for example, a block of cold brass or another appropriate material, as discussed above. The biological sample or other material to be frozen can again be suitably insulated from the heat sink so that an appropriate first rate of cooling occurs.

In a preferred embodiment, material to be frozen is

held within a block of insulating material within the Dewar flask at one or more points spaced between the centre and the periphery of the block. The periphery of the block will be continuously cooled by a cold environment. The centre of the block can receive the brass or other heat sink, which provides the additional rate of cooling necessary for the first rate of cooling.

The way in which the heat extraction means can be constituted is not limited to any of the embodiments discussed above, and may for example be a combination of the particular embodiments described or indeed any other suitable arrangement.

From the above discussion of a preferred embodiment of a passive arrangement, it will be appreciated that the invention also provides means which can be used in conjunction with a dry shipper, liquid nitrogen bath, freezer or any other cold environment, including those discussed above.

According to another aspect of the invention there is provided a device for use in freezing material comprising a liquid, the device comprising a heat sink, insulating means at least partially surrounding the heat sink and means for holding, within the insulating means, material to be frozen, the device being adapted to withstand a temperature at which the material is frozen.

The heat sink may, as before, comprise a block of heat conductive material such as a metal, for example brass.

It may be formed as a core, for example a generally cylindrical core, around which the insulating means may be placed. The core is preferably detachable from the insulating means; the reason for this preference will be discussed below.

The insulating means may be any suitable gaseous, liquid or, preferably, solid insulator. Polystyrene, polytetrafluoroethylene (ptfe) and acetal are acceptable. It will be appreciated that the insulator should have low, but not zero, heat conductivity and/or diffusivity. Polystyrene (unexpanded), for example has a thermal conductivity of 0.04 $W.m^{-1}.K^{-1}$ and a thermal diffusivity of $2.9 \times 10^{-8} m^2.s^{-1}$. The figures for ptfe and acetal are as follows:

	PTFE	Acetal
thermal conductivity $W.m^{-1}.K^{-1}$ @ 23°C	0.24	0.22-0.24
thermal diffusivity $m^2.s^{-1}$	0.74	0.30

The holding means may be any appropriate shape or configuration for holding the material to be frozen. Since at least part of the material will be liquid, the holding means may be adapted to receive a container, for example a straw, ampoule or bag, as discussed above, for the material. Ampoules may be made of glass, plastics or any other suitable material; suitable plastics ampoules include those sold under the trade mark CRYOTUBES. For the case of straws or ampoules to be held in a solid insulating block, the

holding means may simply comprise holes drilled or otherwise formed in the block. Several containers may be received in the same hole. It may be that the insulating block has more than one components, which can is used in a single operation of the device: the components may be stacked, one upon the other, with the cylindrical core being extended appropriately such that it accommodates the entire depth of the stacked insulator block components.

In use, the heat sink (in the preferred embodiment, the brass core) will first be cooled, for example by placing it in a cold environment. The insulating means and the material to be frozen can then be positioned around the heat sink, so that the cold environment at least partially surrounds the insulating means. The material to be frozen will therefore be cooled at the first heat extraction rate by the combined effects of the heat sink and the cold environment until the temperature of the heat sink equilibrates the temperature of the adjacent portion of the insulating means; thereafter, the material to be frozen will be cooled at the second heat extraction rate solely by the effect of the cold environment, the temperature at any time being dependent upon the properties of the cold environment and the thermal properties and dimensions of the insulating means and the heat sink. (The temperature profile is predictable using the computer simulations involved in the design of this piece of equipment, and can be adjusted to suit a required application by varying the parameters considered above.)

The thermal characteristics of the heat sink and the insulating means, the position of the holding means within the insulating means and the nature of the cold environment will be chosen so that heat is extracted from the material to be frozen at the first extraction rate for the appropriate length of time, ie while latent heat is being extracted from the material and the temperature of the material is not substantially falling.

According to a further aspect of the invention, there is provided a method of freezing material comprising a liquid, the method comprising providing material to be frozen within insulating means, at least partially surrounding a cold heat sink with the insulating means, and providing a cold environment at least partially surrounding the insulating means.

The cold environment may be defined by a container which may be well insulated (ie having lower heat conductivity than the insulating means) for example provided by vacuum insulation. The environment may therefore be defined by a Dewar flask or a dry shipper.

A further application of nucleation of aqueous solutions by acoustics would be the controlled, simultaneous nucleation of multiple samples during the cooling phase of freeze-drying. A possible scenario is the freeze-drying of vaccines, where several thousand small glass vials would be cooled, frozen and dried in the freeze-drying apparatus in a single run. Undercooling of the samples during the cooling phase of freeze-drying is, to some extent, inevitable and

without any attempt at synchronised nucleation the ice formation points of individual vials (or other sample container) will vary by several degrees. This will lead to variations in processing time, sample quality as drying begins and inconsistencies in the quality of the completed, dried batch of samples. The problem could be solved if a source of acoutstics was appropriately configured and placed within the freeze-dryer to be used to bring about controlled nucleation and ensure that it coccurred at a required temperature, and uniformly between the samples.

In the foregoing discussion, reference has primarily been made to systems in which liquid water is frozen to ice. However, it will be appreciated that the invention is not limited to water based systems.

Other preferred features of each of the aspects of the present invention are as for the other aspects mutatis mutandis.

For a better understanding of the invention, and to show how it may be put into effect, preferred embodiment of the invention will now be described by reference to the accompanying drawings, in which:

FIGURE 1 is a graph showing how the temperature of a biological sample varies against time as it is cooled through its freezing point;

FIGURE 2a shows a vertical sectional view through a device which is a "passive freezer" embodiment of the invention;

FIGURE 2b shows an exploded perspective view of a further passive freezer embodiment;

FIGURE 2c shows an exploded perspective view of a still further passive freezer embodiment;

FIGURE 3 shows five temperature cooling curves for material cooled in accordance with the invention;

FIGURE 4 shows a temperature cooling curve for plum slices frozen in accordance with Example 1 of the invention and a comparative temperature cooling curve for plum slices frozen by a conventional blast freezing apparatus; and

FIGURE 5 shows a temperature cooling curve for strawberry halves frozen in accordance with Example 2 of the invention and a comparative temperature cooling curve for matched strawberry halves frozen by a conventional blast freezing apparatus.

Referring now to the drawings, Figure 1 illustrates a general problem which is solved by means of the invention. Figure 1 is a graph of time against temperature for a bovine embryo being cooled through its freezing point towards its cryopreservation temperature in liquid nitrogen. The embryo is kept in bovine embryo culture medium plus 10% v/v glycerol as a cryoprotectant, as is conventional, in an 0.25 ml plastic embryo cryopreservation straw. Line A shows the temperature of the cooling environment surrounding the embryo and Line B shows the temperature of the cryporotectant contained in the straw and immediately surrounding the embryo itself. Over time, the environment temperature falls steadily. For the cryoprotectant medium, however, (and, it can be assumed, for the embryo itself, as the temperatures of the cryoprotectant and the embryo will not be expected to be significantly different) the temperature starts to fall steadily, towards and below the melting point (Tm) of the medium containing the embryo. The biological material then supercools until the nucleation point (Tn) is reached. At this point, the water in the material begins to crystallise, and the latent heat of fusion of the water in the sample is released. The temperature of the embryo sample thus increases from Tn to Tm. After the latent heat of fusion has been released, the sample continues to cool, but by this stage the temperature differential between the sample and the surroundings is greater than it previously was. The rate of temperature drop for the sample therefore increases, because of the operation of Newton's law of cooling. The slope of curve B becomes unacceptably steep, which is reflected in damage

occurring to the embryo. In this context, "unacceptable" means the recorded rate of cooling differs (by being more rapid) from the rate recommended or used in conventional practice to achieve successful cryopreservation; an unnaceptable rate is that which could be expected to contribute to serious injury in the frozen sample. This general principle would hold whenever the cooling rate recommended in a published procedure differs significantly from the rate recorded during operation of the protocol: hence the requirement to control cooling rate.

Such difficulties can be avoided by means of the present invention, part of one embodiment of which is shown in Figure 2a, which shows a device 1 which is in accordance with the third aspect of the invention and which is adapted to be placed in a cold environment such as in a Dewar flask or dry shipper containing liquid nitrogen.

The device 1 comprises a vertically arranged, circular-sectioned cylindrical brass core 3, which is 140mm long and 27mm in diameter. The core 3 is provided at its bottom end with a spigot 5 for location in a corresponding socket in a bevelled, centrally located boss 7 integral with a base plate 9. The base plate 9 and boss 7 are constructed from laminated polystyrene. The base plate 9 is in the form of a disc 200mm in diameter and 20mm thick. The boss 7 has a minimum diameter of 27mm, to correspond with the brass core 3, a height of 20mm, and is bevelled outwardly towards the base plate 9 at 45°. In use, the brass core 3 is firmly attached to the boss 7 and base plate 9.

An insulating block 11, generally in the form of a hollow circular-sectioned cylinder is configured to slide and fit over the brass core 3 and to seat snugly in the boss 7 and base plate 9. The insulating block 11 is also constructed from laminated polystyrene and it has a maximum height of 180mm and a diameter of 200mm. Its hollow has a diameter of 2.7cm to correspond with the brass core.

A first series of twelve holes 13 are formed in the insulating block 11. They extend vertically downwardly, parallel to the axis of the brass core 3 and are symmetrically arranged about the core's axis. Each hole 13 in the first series is 3mm in diameter and extends down from the uppermost surface of the cylindrical block 11 to a depth of 140mm. The axis of each of the holes 13 lies 35mm from the axis of the brass core 3 or 21.5mm from the periphery of the brass core 3.

Second, third and fourth series of twelve holes lie, in register, radially outwardly from the first series; representative holes are indicated by reference numerals 15, 17 and 19, respectively. The axis of the holes of the second series 15 lie 50mm radially outwardly from the axis of the brass core 3, and the corresponding distances for the third and fourth series 17 and 19 are 65mm and 80mm; otherwise the holes of the second, third and fourth series 15, 17 and 19 are as for the first series 13.

The purpose of each series of holes 13, 15, 17 and 19 is to hold plastics straws (not shown) conventionally

used for the cryopreservation of mammalian embryos and gametes. Such straws are available from IMV, L'Aigle, France, and are internally coated with cholesterol, as taught in EP-A-0246824. Instead of coating straws (or any other container) with cholesterol, crystals of an appropriate nucleator, including cholesterol, can be added to the contents. Appropriate nucleators are available from Cell Systems Limited under the trade marks CRYOSEED or XYGON.

On top of the insulating block 11, and covering the top of the brass core 3 and the first to fourth series of holes is an insulating cover plate 21 in the form of a disc of 200mm diameter to correspond to the insulating block 11. The cover plate 21 is constructed of laminated polystyrene and is 20mm thick.

In use, the brass core 3 and base plate 9 are first placed in a cold environment, for example in a dry shipper. (A dry shipper is a well insulated container resembling a large Dewar flask lined with absorbent material containing liquid nitrogen; because the nitrogen is absorbed, there is little or no free liquid in the shipper.) The brass core 3 is allowed to equilibrate with the cold environment, whereafter the insulating block 11, containing twelve straws in the first series of holes 13, each containing a bovine embryo, is positioned round the brass core 3 to seat on the base plate 9. The cover plate 21 is then placed on the insulating block 19, and the device 1 is left to cool.

Initially, the straws are cooled both by the influence of the brass core 3 and by the cold environment. This combined action provides a relatively high rate of heat extraction from the embryos. The cooling curves of five samples of cooling medium for bovine embryos in the first series of holes 13 are shown in Figure 3. (The embryos are in cryopreservation straws containing bovine embryo culture medium plus 10 % v/v glycerol as a cryoprotectant.) The first heat extraction rate is applied while the water is supercooling, shown at region C of the curve. The temperature of the sample drops below the melting point (Tm) and supercooled slightly to the nucleating point (Tn). The nucleating temperature is not far below the melting point, because of the presence of the cholesterol ice nucleator within the straws. However, when the temperature reaches the nucleating point (Tn) the sample temperature rises as shown at D to the melting point (Tm). By the time the temperature of the embryos begins significantly to drop again, the brass core 3 has substantially equilibrated with the embryos and the intervening material of the insulating block 11. Therefore, the continued heat extraction is solely towards the periphery of the insulating block 11, and so the rate of heat extraction from the samples is lower. The slope of the graph at E is therefore acceptably smooth and no too steep and no damage results to the embryos, which can then safely be allowed to cool to the temperature of the cold environment (-80°C). In the temperature range -25° to -30°C, the average rate of cooling was found to be 0.32°C/min with this configuration.

Figure 2b shows a further embodiment of a passive freezer, broadly similar to that shown in Figure 2a, but including a handle assembly 101 and locating lugs 103 on an insulating block 105 adapted to extend through a cover plate 107 and to engage apertures in a locating disc 109 of the handle assembly 101. A locating lug 111 on the cover plate 107 locates in a spigot 113 of the handle assembly 101. The insulating block 105 is made of acetal and has sample placement holes 106 adapted to receive 2.5ml ampoules for cryopreservation of, for example, mammalian cell lines. The insulating block 105 is seated on a bevelled boss 115 on a base 117 and surrounds a brass core 119. All components other than the brass core are made of acetal. Salient dimensions of the device of Figure 2b are as follows:

ACETAL CONSTRUCTION
cryo-ampoules [c2.5ml]

Component	DIMENSION [mm] Diameter:Depth/height
1 Lid 107	200 : 40
2 Block 105	200 : 140
3 Locating lugs 103 [2]	15 : 52
4 Brass rod 119	57 : 140
5 Base 117	200 : 20
Machined holes	
6 Sample placement holes 106	13 : 50
7 Countersink for boss 115	5
8 Centre of sample placement hole 106 to perimeter of block 105	44
9 Centre of locating lug 103 to perimeter of block 105	22.5
10 Hole for brass rod 119	57 : 140

Note 1: the height of the locating lugs 103 does not include threaded portion inserted into block - dimensions not critical

Note 2: the height of the brass rod 119 does not include locating lug on base - dimensions not critical

Note 3: the base 117 has three small acetal feet mounted, equally spaced, at the periphery. Feet 5mm high x 5mm diam. Size of boss to locate brass rod and block not critical.

This construction, when used in conjunction with a liquid nitrogen-containing dry shipper, allows a cooling rate of -1°C/min.

A different embodiment, essentially similar in construction to that shown in Figure 2b but for use in connection with cryopreservation straws (eg for bovine embryos), has the acetal component parts replaced with PTFE parts. The salient dimensions are as follows:

PTFE CONSTRUCTION
plastic straws
[0.25/0.5ml]

Component	DIMENSION [mm] Diameter : Depth/height
1 Lid 107	200 : 20
2 Block 105	200 : 160
3 Locating lugs 103 [2]	35 : 10
4 Brass rod 119	22 : 160
5 Base 117	200 : 20
Machined holes	
6 Sample placement holes 106	3 : 133
7 Countersink for boss 115	5
8 Centre of sample placement hole 106 to perimeter of block 105	63
9 Centre of locating lug 103 to perimeter of block 105	30
10 Hole for brass rod 119	22 : 160

Note 1: the height of the locating lugs 103 does not include threaded portion inserted into block - dimensions not critical

Note 2: the height of the brass rod 119 does not include locating lug on base - dimensions not critical

Note 3: the base 117 has three small acetal feet mounted, equally spaced, at the periphery. Feet 5mm high x 5mm diam. Size of boss to locate brass rod and block not critical.

This construction, when used in conjunction with a liquid nitrogen-containing dry shipper, again allows a cooling rate of -0.3°C/min.

Figure 2c shows a still further embodiment of a passive freezer. The construction is a modification of that shown in Figure 2b, and like components have been given the same reference numerals. The principal difference is that in the Figure 2c construction the insulating block 105 has been replaced with two half height blocks 105a and 105b; this allows for more of ampoules to be present (up to 15). Salient dimensions of the device of Figure 2c are as follows:

ACETAL CONSTRUCTION
cryo-ampoules [c2.5ml]

Component	DIMENSION [mm] Diameter:Depth/height
1 Lid 107	200 : 40
2 Block 105a	200 : 70
3 Block 105b	200 : 70
4 Locating rods 103 [2]	15 : 123
5 Brass rod 119	57 : 120
6 Base 117	200 : 20

Note 1: the height of the locating lugs 103 does not include threaded portion inserted into block - dimensions not critical

Note 2: the height of the brass rod 119 does not include locating lug on base - dimensions not critical

Note 3: the base 117 has three small acetal feet mounted, equally spared, at the periphery. Feet 5mm high x 5mm diam. Size of boss to locate brass rod and block not critical.

Machined holes	
7 Sample placement holes 106	13 : 50
8 Countersink for boss 115	5
9 Centre of sample hole 106 to perimeter of block 105	44
10 Centre of locating lug 103 to perimeter of block 105	22.5
11 Hole for brass rod 119	57 : 120

It must be noted that the configuration described here in detail are only a few of a great number of possible configurations, depending upon the cooling rate required and the type of sample holder (for example straw or ampoule) to be cooled.

The variables can be:

i the diameter of the insulator (although in practice it may be convenient to use a standard diameter for a range of products for manufacturing and marketing reasons);

ii the depth of the insulating block;

iii the diameter of the metal core;

iv the number, size and placement of the holes for the samples; and

v the materials of the insulating block and metal core.

The invention will now be illustrated by the following examples, which relate to active, as well as passive, systems. Unless otherwise stated, all examples of active systems in accordance with the invention (ie those active examples other than comparative examples) were carried out in a PLANAR KRYO 10/16 controlled rate freezing machine. (The expression PLANAR KRYO 10/16 is a trade mark). Temperature was measured with T type thermocouples connected to a SQUIRREL data logger (1200

series). (The word SQUIRREL is a trade mark.) Data were transferred to an IBM-compatible computer for storage and analysis. In order to compare different treatments, the time the sample is at the latent heat plateau, defined here as the exotherm time (ET), is used; this is further defined by the final temperature eg ET^{-5} or ET^{-10} being the time from the exotherm to -5°C or -10°C respectively. Application of acoustics was either from a Branson model 250 sonicator operating at 20kHz, a Branson Model 2200 ultrasonic cleaner, a Lucas-Dawe series 6266 immersible transducer, a Telesonics tube resonator type TR connected to a ultrasonic generator type USR-20 (20kHz) or a HILSONICS acoustic driver, model IMG 400 (Hilsonic Ltd, Merseyside, England).

Example 1

This example shows that plums freeze better when using an efficient latent heat removal protocol of the invention, even in the absence of acoustics, as compared to conventional methods. Korean dark skinned plums (Tesco foodstores) were sliced into 4.5mm slices and were frozen by a method in accordance with the present invention. For comparison purposes, plum slices were also frozen by conventional methods. The methods used are as follows.

1. Slices were frozen by a method in accordance with the invention. The initial environment temperature was -75°C, which was held for 2 minutes. The environment temperature was then warmed to -30°C at 10°C/min. The temperature reduction in the plum slice was significantly

faster than in the blast freezer treatment (2, below), with a measured exotherm (ET-10) of 80 seconds (Figure 4).

2. (This is a comparison method.) Slices were placed in a commercial blast freezer operating at -40°C; the measured exotherm (ET^{-10}) was 554 seconds (Figure 4). They were then transferred to a commercial deep freeze operating at -20°C.

3. (This is a comparison method.) Material was immersed directly into liquid nitrogen and transferred to a commercial deep freeze. The sample cooled quickly through its exotherm; however the final temperature attained was below -100°C.

Sensory evaluation of frozen/thawed material was made against fresh plum slices. Frozen plums were removed from the freezer 45 minutes before evaluation and laid on a plate with cling film to cover them. The plums were placed on paper plates before panellists singly, on demand, according to a statistically randomised design. The panellists were instructed to assess the flesh only and to discard the skin of the fruit. Malvern water was used as a mouth wash between samples. 24 replicate tastings of each sample were carried out. The assessment took place under purple lighting to disguise any colour differences.

Results

Adjusted mean scores for the whole trial are shown

below; the scores are on a scale of 1-10.

Texture:	1	2	3	4
Firmness	5.46	3.46	6.08	7.83
Wetness	6.46	7.75	5.67	2.92
Crispness	5.42	4.00	6.33	6.79
Fibrous/Chewiness	6.25	5.29	6.71	7.42
Particulateness	5.25	4.71	5.75	6.88
Juiciness	6.92	7.46	6.08	3.79
Flavour:				
Overall strength	6.33	6.88	6.04	3.75
Sweetness	4.79	4.88	4.38	3.63
Sharp/Acidic	4.79	4.71	5.00	2.96
Bitterness	2.83	2.96	2.88	2.25

Key: 1 = Present invention; 2 = Blast frozen; 3 = Liquid nitrogen; 4 = Fresh

Discussion

Present invention vs. Fresh.

The fresh sample is significantly firmer, drier, more fibrous/chewy than the sample frozen by the invention. In flavour terms the fresh sample is lower in flavour overall, less sweet and less sharp/acidic than the plums frozen by the invention.

Present invention vs. blast freezing.

The plums frozen by the present invention are significantly firmer and more fibrous/chewy than the

blast frozen plums. The remaining parameters show no significant differences.

Present invention vs. liquid nitrogen freezing.

There were no significant differences for any parameters.

Example 2a

This example shows that strawberries freeze better when using an efficient latent heat removal protocol of the invention, even in the absence of acoustics, as compared to conventional methods. Spanish class 1 strawberries (Sainsburys Foodstores) were halved and frozen by the following methods:

1) Simulation of blast freezing in a Planar controlled rate freezer, with a rate of cooling of the gas temperature of 1°C/min. The measured exotherm was 660 seconds (Figure 5).

2) Frozen by a method in accordance with the invention. The initial environment temperature was -50°C for 7 minute with rewarming at 10°C/minute to -30°C. The measure exotherm in the matched strawberry half to treatment 1 was 280 seconds (Figure 5).

3) Strawberries were frozen by immersion into liquid nitrogen.

Results.

Following freezing in liquid nitrogen many strawberries fractured. Strawberries blast frozen and immersed in liquid nitrogen displayed significant leakage of cellular contents. For those frozen by the present invention leakage was less pronounced and the strawberries were significantly firmer. The exudate was less pigmented than following blast freezing or liquid nitrogen freezing, clearly demonstrating that less intracellular damage occurred following the current method.

Sensory evaluation of the frozen/thawed material was made against fresh strawberries. Frozen strawberries were removed form the freezer 45 minutes before evaluation. 25 independent replicate tastings of each sample were carried out.

Texture:

	Treatment		
Rating	1	2	3
Excellent	-	-	-
Very Good	0	3	1
Good	2	6	2
Fairly Good	3	7	10
Moderate	12	6	9
Poor	7	3	2
Very Poor	1	0	1

Key: 1 = Blast Freezing; 2 = Invention; 3 = liquid N_2

Flavour:

	Treatment		
Rating	1	2	3
Excellent	-	-	-
Very Good	-	1	3
Good	3	7	5
Fairly Good	4	5	3
Moderate	8	8	4
Poor	5	2	8
Very Poor	5	3	2

Key: 1 = Blast Freezing; 2 = Invention; 3 = liquid N_2

There appeared to be little effect of storage time, within the range of from 1 to 30 days, on the quality of the material frozen by the method in accordance with the present invention.

Both the type of strawberry and the degree of ripeness also determined the quality on thawing; the observations here are not intended to be exclusive but rather to be a guide to the trends observed. The best results were obtained with slightly under-ripe class 1 Spanish strawberries. Poorer results were obtained with riper class 1 strawberries of the same type. Good results were achieved with slightly under-ripe class 2 Carmel strawberries (from Israel). With ripe class 1 Carmel strawberries and class 1 Kenyan strawberries (Sainsburys Foodstores) poorer results were obtained. It must be emphasised that with such riper starting material the results following the method in accordance

with the present invention outlined above was always superior to blast freezing or liquid nitrogen freezing of the same material.

Example 2b

This example shows that even better results are obtained when strawberries are frozen using an efficient latent heat removal protocol, with the application of acoustics. Strawberries (Californian guadalupe) were obtained in bulk from a retail outlet and sorted to discard all over- or under-ripe material. The selected strawberries were washed and then halved. The separated halves of each fruit were collected together to provide two populations of 280, essentially matched strawberry halves.

The strawberries were frozen in batches of 70 halves.

A 12"x12" (30.5cm x 30.5cm) acoustic plate (22.5 kHz, 220V, Hilsonic Ltd, Birkenhead, UK) was precooled to -70°C in a CryoMed 2700 freezer and the strawberry halves loaded on to it, which resulted in a temperature rise to -50°C. The material was cooled according to the following protocol: (1) providing an initial environment temperature at -58°C for one minute; (2) warming at 10°C/minute to -48°C.

Sample temperature was monitored using type T thermocouples embedded in the mid-point of representative strawberry halves, connected to a microprocessor data-logger (Grant Instruments, Cambridge, UK). When the samples reached -20°C they

were transferred to storage at -30°C for 5 days. Samples were thawed by exposure to room temperature for 90 minutes before sensory evaluation.

When an acoustic treatment was applied a pulse of 2 sec every 30 sec was used throughout the entire cooling cycle.

Subsequently thawed strawberries were subjected to a sensory evaluation panel, with the following results:

Characteristic	- acoustics	+ acoustics	sig. dif. in mean scores due to acoustic treatment
Berry colour 1=dull red 9=bright red	5.6	6.2	nsd
Free liquid on plate 1=small amount 9=large amount	4.3	3.4	0.01
Firmness 1=soft 9=firm	3.2	4.5	0.01
Mushiness 1=not mushy 9=very mushy	6.2	4.9	0.01
Overall appearance 1=dislike extremely 9=like extremely	5.4	6.4	0.05
Overall Texture 1=dislike extremely 9=extremely	4.2	5.5	0.01
Overall flavour 1=dislike extremely 9=like extremely	5.0	6.0	nsd
Overall opinion 1=dislike extremely 9=like extremely	4.6	5.8	0.10

Example 3a

This example shows that a blanched vegetable, celery, freezes better when using an efficient latent heat removal protocol of the invention, as compared to conventional methods, and that even better results are obtained in the additional presence of acoustics.

Celery was obtained from a retail outlet. Celery samples were cut into 0.6cm (¼ inch) pieces, and 250g were blanched per run at 90°C (190°F) for 2 minutes. There was a loss of 10% material on blanching. The samples were rinsed with cold water to bring them to room temperature (20°C). The celery samples were then frozen in accordance with the invention using the following protocol:

(1) The initial environment temperature was maintained at -75°C for 2 minutes;

(2) The environment temperature was then warmed to -30°C at 10°C per minute. This protocol was followed with and without the application of acoustics. When acoustics was applied, an ultrasound frequency of 22.5kHz was used, and the power level was 220 watts, applied over an area of 929cm^2 (144 square inches), resulting in a power level of 0.24W/cm^2. The ultrasound was not applied continuously, but rather was applied for 3 seconds every 30 seconds.

As a control, the blanched celery was also blast frozen at an environment temperature of -40°C. The samples were removed when they reached -30°C. After treatment,

some of the frozen celery samples were stored at -30°C and some were subjected to a standard temperature abuse protocol.

The resulting samples were evaluated in a balanced, sequential order by a tasting panel consisting of 42 panelists, who had been pre-screened to have a positive attitude towards evaluating frozen celery slices that had been thawed. A serving consisted of 6 slices of celery that had undergone a given treatment. The celery had been thawed at ambient temperature for 60 minutes prior to serving; this was sufficient to eliminate any ice crystals, yet still to be slightly chilled. The panelists were instructed to evaluate all slices having undergone a given treatment before rating the attributes, so that the rating would reflect the majority of slices.

The results showed that the efficient latent heat removing protocol in accordance with the ivention resulted in better firmness, less mushiness and a better overall impression of freshness of flavour than the control, blast-frozen samples. Further, when acoustics was also applied, it was not only found that the samples offered textural advantages over the control samples, but it was also found that they held up better under temperature abuse than the control samples. An additional advantage of the invention displayed was the reduction in the time taken for the sample temperature to be reduced from ambient to the storage temperature (-30°C). Using prior art blast freezing techniques, the time taken to reach -30°C is in the order of 20 minutes. Using an efficient latent

heat removal protocol in accordance with the invention, this time is reduced to about 8.2 minutes. A further improvement to about 5.2 minutes, is seen with the additional application of acoustics.

Example 3b

Celery sticks were purchased from a local supermarket (Tesco foodstores), washed and cut into 1cm sections. They were blanched for 3 minutes at 80°C, then flushed with cold water. Samples were frozen according to three methods:

(1) Simulated blast freezing (Planar Kryo 10 set at -40°C);

(2) According to the invention, using an initial environment temperature of -50 °C, with a hold time of 8 minutes, and then warming to -20°C at a rate of 10°C/min.

(3) As in (2) with the addition of acoustics supplied from a 20cm x 20cm plate equilibrated at -50°C (25kHz, 260W power, 2 seconds per 30 seconds pulse time).

On thawing, texture of the three samples was assessed according to a subjective assay, the results of which were as follows:

Scored 0-5 (0=poor, 5=excellent)

The average taste panel scores for each treatment were:

Treatment (1) - 2.5
Treatment (2) - 3.0
Treatment (3) - 4.0

Example 4a

Small new potatoes of less than 4 cm in diameter (Sainsbury's Foodstores) were frozen by a number of treatments, as described below, and evaluated on thawing. Potatoes were neither cooked nor blanched before freezing.

1) The potatoes were 'blast frozen' as for strawberries in Example 2a above; on thawing the potatoes were very soft, leaked cell water and were considered unacceptable after cooking.

2) The potatoes were frozen by liquid nitrogen immersion; they invariably fractured during freezing.

3) The potatoes were frozen by a method in accordance with the present invention by (1) providing an initial environment temperature of -80°C for 1 minute, (2) warming at 10°C/minute to -20°C. On thawing, the potatoes were intact and retained their original texture with no leakage. On boiling, the potatoes were acceptable.

Example 4b

Small new potatoes (3-5cm length, var. M.Bard, Tesco foodstores), were cooked in boiling water for 15 minutes, then flushed with cold water until cool. 200g batches were frozen to -30°C by the following methods;

(1) Simulated blast freezing (-40°C) in a Planar Kryo 10 freezer.

(2) According to the invention, using a Planar Kryo 10 freezer. The initial temperature was -50°C, which was held for 6.5 minutes; the temperature was then allowed to rise a a rate of 10°C per minute until -20°C was reached.

(3) As (2) with the addition of ultrasound, supplied over 20cm x 2cm at 360W, 25kHz, and various pulsing lengths, as described below.

The lengths of latent heat plateaus in the various treatment were measured. Following thawing, batches were assessed by a taste panel, and quantitative drip loss by halving tubers, wrapping in gauze in a funnel, and placing a 3lb (1.36kg) weight on the sample for 20 minutes. Smears of sample material were mounted on a microscope slide, and observed using light microscopy.

The results are given below.

(1) Lengths of latent heat plateaus (LHP's) in various cooling treatments, were as follows:

		LHP length (minutes)
Treatment 1		8
Treatment 2		6.5
Treatment 3	2s in 15s	7.0
	2s in 10s	5.0
	2s in 5s	4.0

According to sensory evaluation, the treatments were ranked for texture in the following order;

Treatment 3 2s in 5s > Treatment 3 2s in 10s > Treatment 3 2s in 15s > Treatment 2 > Treatment 1.

(2) Fluid extrusion.

Treatment	Fluid Extruded
1	11
2	9
3 2s in 40s	7

(3) Microscopy

Cells from Treatments 1 and 3 were compared. Blast frozen cells showed a loss of organized cell structure and contents, with extensive folding of the cell membrane. By contrast, cells frozen by Treatment 3 (acoustics), showed good retention of cellular integrity, and less folding of the cell membrane.

Example 5

Two types of asparagus obtained from Sainsbury's Foodstores, which were Peruvian and Thai in origin respectively, were frozen by a number of methods as described below and evaluated following steaming of the thawed product.

1) Both types of asparagus were blast frozen as described in Example 2a. The subsequently thawed product had poor taste and texture and scored 4/20.

2) Both types of asparagus were frozen in liquid nitrogen. The spears fractured and, on thawing, had very poor taste and texture; they scored 2/20.

3) Both types of asparagus were frozen by a method accordance with the present invention by (1) providing an initial environment temperature of -80°C for 1 minute and (2) rewarming to -20°C at 15°C/minute. On thawing, the taste of the spears was improved, as was their texture on cooking; they scored 10/20.

Example 5b

Raw asparagus spears (produce of Thailand, purchased at Sansibury's foodstore) were trimmed to 6 inch (15cm) lengths, and frozen by:

(1) Simulation of blast freezing in a Planar controlled-rate freezer, set at -40°C.

(2) Frozen in a KRYO 10 series chamber Model 10-16 controlled rate freezer by Planar Biomed, Sunbury on Thames, England, in accordance with the invention optimised by computer modelling. The initial environment temperature was -50°C, which was held 12 minutes, and the temperature was then increased at a rate of 10°C per minute until -20°C was reached.

(3) Frozen as in (2) with addition of acoustics (22.5kHz, 360W power, 2 seconds per 20 seconds). Acousrtics was supplied by a HILSONIC acoustic driver model IMG 400 (Hilsonic Ltd, Merseyside, England) coupled through an ISOPAR M liquid filled chamber to an 8" x 8" (20cm x 20cm) plate forming the floor of the freezer chamber. Following freezing, the samples were thawed to ambient temperature over 6 hours. The spears werethen cooked for 4 minutes in boiling water, and the three frozen treatments compared with an unfrozen sample using a taste panel.

The panel recorded average scores (0 - 5, 0=poor, 5=excellent):

Unfrozen - 5
Method (1) - 1.5
Method (2) - 2.5
Method (3) - 3

Example 6a

Single cream is an example of a oil in water emulsion. Single pasteurised cream was obtained from Sainsbury's Foodstores. Following freezing and thawing of this

product, separation of the cream solids from the liquids occurs. Freezing damage may be assessed by the loss of liquid through a small mesh filter. 10 ml aliquots were placed in glass universals and frozen by a variety of methods, as described below:

1) Blast freezing, as described in Example 2: on thawing the cream is discoloured yellow, curdled. The liquid loss is 34%;

2) Liquid nitrogen immersion, as described in Example 2a; on thawing the cream does not visually separate but becomes very viscous. The liquid loss is 12%; and

3) Freezing by a method of the present invention, with an initial environment temperature of -80°C for 1 minute, followed by warming at 15°C/minute to -20°C. On thawing the cream does not visually separate; there is an increase in viscosity but not as pronounced as with liquid nitrogen freezing. The liquid loss is 10%.

4) Freezing as for method (3) except that ultrasound was applied for 0.1 seconds for every 1°C cooling of the cream from 0 to -20°C. This combination of acoustic nucleation and efficient removal of latent heat consistently, in five independent trials, further reduced the drip loss by 10-16% of that observed in method (3).

It can be seen, therefore, that the present invention gives results which are appreciably better than blast

freezing and which are also better than the more expensive and relatively inconvenient process of freezing by liquid nitrogen immersion.

Example 6b

Single cream (Tesco foodstores) was divided into 100ml batches, either in freezer bags supported by metal frames or in metal moulds.

The cream was frozen according to the following methods:

(1) Simulated blast freezing (-40°C) using a Planar Kryo 10.

(2) According to the invention, involving rapid freezing by immersing samples in a Planar Kryo 10 controlled rate freezer initially at -80°C (hold 10 minutes), then warmed to -20°C at 10°C per minute, with the addition of acoustics throughout the cycle (300W over 20cm x 20cm, 22kHz, 2 seconds every 60 seconds pulsing).

(3) According to the invention, using a Planar Kryo 10 freezer at -50°C, holding 15 minutes, with the addition of acoustics throughout the cycle as in (2).

Sensory analysis of the three tratments post-thaw, indicated as follows:

(1) Separation of the cream had occurred, resulting in liquid loss, very grainy, and buttery tasting.

(2) Very good texture, no fluid loss.

(3) No fluid loss, but texture not as good as in (2).

Example 7

Mayonnaise is an example of a water in oil emulsion. Commercial mayonnaise, such as Hellman's, appears to be stable following a wide range of freezing methods. This probably reflects the degree of physico-chemical stabilisation of the product. Home-prepared mayonnaise and non-stabilised commercial mayonnaise such as Kite wholefood mayonnaise separate following freezing and thawing. Such mayonnaises were frozen in 10 ml aliquots in glass universals by the following methods:

1) Blast freezing, as in Example 2a; total separation of the oil occurred on thawing;

2) Liquid nitrogen immersion, as in Example 2a; total separation of oil occurred on thawing; and

3) Freezing by a method in accordance with the present invention, in which the mayonnaise was cooled at 20°C/minute from 0°C to -50°C, held at -50°C for 2 minutes, warmed at 15°C/minute to -20°C. On thawing, there was good retention of texture with little or no separation of constituents.

Example 8

Prepared prawn and mayonnaise sandwiches were obtained from Tesco and Sainsbury's Foodstores and singly frozen by a variety of methods, as follows:

1) Blast freezing as described in Example 2a; on thawing there was a total separation of the mayonnaise: the oil component seeped through the lower slice of bread and the product was totally unacceptable;

2) Liquid nitrogen immersion as described in Example 2a; fracturing of the sandwich occurred and on thawing there was total separation of mayonnaise as in (1) above;

3) Freezing by a method in accordance with the present invention, in which each sandwich is cooled at 20°C/minute to -50°C, held isothermally at that temperature for 30 minutes and then warmed at 10°C/minute to -20°C. On thawing the product was acceptable. There was little or no separation of the mayonnaise, good retention of prawn quality and no fracturing of the bread.

Example 9

Fillets of fresh Scottish smoked salmon (Sainsbury's foodstore) were frozen according to two methods:

(1) Simulation of blast freezing in a Planar Kryo 10 controlled-rate freezer st at -40°C.

(2) In accordance with the ivnention, using thermal modelling and ultrasonics application. The initial environment temperature was -50°C, which was held for 4 minutes, and the temperature was increased at a rate of 10°C per minute until -20°C was reached. Ultrasonic acoustics was supplied at 360W over 20cm x 20cm, 22.5kHz and 2 seconds per 40 seconds pulsing.

Following thawing, samples were tested by a panel for texture and taste. The panel recorded average scores of:

Unfrozen : 5
Method (1) : 1
Method (2) : 3

(0-5, 0=poor, 5=excellent).

Example 10

25ml ice pops (similar to sorbets) were obtained from a local supermarket (Tesco Foodstores), and frozen according to two methods;

(1) By processing according to the invention by holding first at -50°C for 5 minutes and then increasing the temeprature at 10°C/min until -20°C was rached in the sample, as detected by a thermocouple.

(2) As (1), with the addition of ultrasound delivered from a 20cm x 20cm plate equilibrated at

-50°C, powered by a 260W, 22.5kHz generator, 2 seconds per 40 seconds pulsing. There results were as follows: Cooling profiles in the two treatments varied, with acoustic treatment considerably reducing latent heat plateaus, and freezing time to -20°C. An assessment of crystal size by eye indicated smaller ice crystals were present in the sample frozen with acoustics compared to the sample frozen without. In addition, the ice pops frozen with acoustics were harder to the bite and crispier in texture than those without acoustics.

Example 11

Cream cheese (Kraft General Foods) was sliced into ½ inch (1.3cm) cubes, and samples frozen according to the following methods:

(1) Simulated blast feezing in a Planar Kryo 10 controlled rate freezing apparatus held at -40°C;

(2) According to the invention, again using a Planar Kryo 10 apparatus but using a hold time at -50°C for 5 minutes then warming at 10°C/min to a temperature of -20°C.

(3) As (2), with the addition of ultrasound, supplied at 360W over 20cm x 20cm, 25kHz, 2 seconds per 30 seconds pulsing.

When thawed, the samples were analysed by a taste panel on a 0-5 ranking (0=poor, 5=excellent). The average scores were:

Unfrozen : 5
Method (1) : 3
Method (2) : 3.5
Method (3) : 4.0

Example 12

Lean beef was obtained from a local butcher and sliced into approximately 1" (2.5cm) cubes. Four samples of 375g each were frozen according to the following methods:

(1) Using a -20°C chest freezer

(2) Simulation of blast freezing (-40°C, Planar Kryo 10).

(3) According to the invention, in a Planar Kryo 10 controlled rate freezer kept initially at -50°C for 15 minutes and then warmed at a rate of 10°C/min until the temeprature reached -20°C. Acoustics (360W over 20cm x 20cm, 25kHz, 2 seconds per 30 seconds pulsing) was supplied.

Following incubation at -20°C overnight, samples were thawed, and fluid loss from the samples assayed over 6 hours.

(1) 14ml
(2) 3ml
(3) 2.5ml

Example 13

This example demonstrates that acoustics imporve an otherwise conventional blast freezing process.

Belgian strawberries were purchased from a local supermarket (Tesco Foodstores), washed, halved and divided into 100g batches.

Batches were frozen according to the following methods:

(1) Simulation of blast freezing in a Planar Kryo 10 controlled-rate freezer, set at -40°C.

(2) As (1), with the additionof a 20cm x 20cm ultrasonics plate equilibrated at -40°C, supplied by an external generator with 360W, 25kHz, with pulsing of 2 seconds every 30 seconds, 2 seconds every 60 seconds and 2 seconds every 120 seconds.

(3) As (2) with 260W power.

Following freezing, samples were assayed for drip loss over a 6 hour period.

The results obtained were as follows:

Freezing Method	Drip loss (ml)	
	260W power	360W power
(1)	12	14
(2) 2s in 30s	13	18
2s in 60s	10	15
2s in 120s	12	12

These results indicate that improved freezing can be obtained when blast freezing/acoustics are combined, providing pulsing intervals are optimized.

Example 14a

This example demonstrates that acoustics improves an otherwise conventional chest freezing process.

Honeydew melons wee frozen to -20°C according to two methods:

(1) In a chest freezer set at -20°C.

(2) On a 20cm x 20cm ultrasonics plate equilibrated at -20°C powered by a generator providing 22.5kHz frequency, 260W power, at on/off intervals of 2 seconds every 40 seconds.

(3) As (2) with a fluid-filled plate, incorporating a glycol-filled layer.

Upon thawing, the treatments were assayed by a taste panel, which scored for texture on a range of 0 (poor) - 10 (excellent).

Treatment (1) 2
Treatment (2) 4.5
Treatment (3) 3.5

Example 14b

Honeydew melons (Tesco Foodstores) were halved and, using a 3cm diameter scoop, samples were removed, mixed and 200g portions frozen by the following methods:

(1) Simulation of blast freezing in a Planar Kryo 10 controlled-rate freezer, set at -40°C.

(2) Frozen in accordance with theinvention. The environment temperature was initially -50°C, with a holding time of 16 minutes, and the temperature was raised at a rate of 10°C per minute to -20°C.

(3) Frozen as in (2), with the addition of acoustics (22.5kHz, 260W over 20cm x 20cm, 2 seconds per 30 seconds).

Following freezing, the samples were maintained at -20°C overnight, then thawed for 6 hours. The fluid lost from each sample was recorded:

(1) 31mls
(2) 15mls
3) 13mls

Example 15

A typical ice cream mix without preservatives was frozen in a chest freezer at -50°C with and without the

application of acoustics. 13 samples (25 to 27ml) were placed in stainless steel cylindrical moulds (length 12cm, mean diameter 2.2cm) and immersed in a 30% w/v solution of calcium chloride in a Branson (Shelton, Connecticut, USA) Model 2200 ultrasonic cleaner. The ultrasonic cleaning bath was placed in the chest freezer and the bath solution was maintained at -40°C. For the samples under test, acoustics was applied at 70 to 80% of the maximum power level (120W) at a frequency of 47kHz. The frequency was pulsed for 45 seconds every 30 seconds. The samples were removed when a temperature of -30°C was reached. The control and experimental samples of the frozen ice cream mix were divided into halves, with one part being stored at -30°C and the other being subjected to accelerated thermal abuse.

A significant improvement in quality was observed in a blind taste test for the ice cream that had been subjected to acoustics during the freezing process. Additionally, the time taken to reach -30°C was significantly less, when acoustics was applied. Freezing could therefore be achieved more rapidly with the application of acoustics.

Example 16

This example demonstrates that the acoustics aspect of this invetion has application during the cooling phase of a freeze-drying (lyophylisation) operation.

0.5ml of distilled water was placed in each of 20 conventional glass freeze-drying vials and cooled to

-4°C without freezing. The vials were placed on a precooled (-5°C) 20cm x 20cm acoustic plate (Hilsonic Ltd) and immediately subjected to 2 seconds of 25kHz acoustics at 320W. The contents of each of the vials nucleated instantly, demonstrating the feasibility of nucleating undercooled aqueous or other solutions in glass vials, using an acoustic source that was configured such that it could also be used as the shelf upon which the vials were standing.

Example 17 - Bacterial Cells

Bacteria were harvested from culture slopes in 10ml of nutrient broth + 10% v/v glycerol and the resulting suspended bacterial population measured into 1ml aliquots in polypropylene CRYOTUBES [2ml]. CryoSeeds™ cholesterol crystals [Cell Systems, Cambridge] were added to each tube to ensure reproducible ice nucleation.

The tubes were transferred either to a Planar Kryo 10 conventional programmable freezer [Planar Products, Sunbury on Thames, Middx] or to a passive freezing device as described above in relation to Figure 2b and configured to be cooled at 1°C per minute. The tubes were cooled to -70°C, when they were removed and plunged into liquid nitrogen. Samples temperatures were monitored using a Type T thermocouple/electronic thermometer combination with the probe immersed in one of the samples.

The tubes were thawed by immersion in water at 25°C and the samples spirally-plated onto nutrient broth to provide a viable cell count.

Bacterium	% viable cells [means of duplicate cultures]	
	Planar freezer	Passive freezer
Escherichia coli	82.45	82.70
Staphylococcus aureus	80.70	81.45
Neisseria meningitidis	63.85	59.45
Haemophilus influenzae	59.50	70.65
Vibrio cholerae	75.70	72.45

The results show that the passive freezer of this invention enables good results to be obtained even with a small and portable piece of equipment.

Example 18 -Bovine embryos

Bovine embryos at the 4-cell stage of development were incubated in ovum culture medium + 10% v/v glycerol and then loaded individually into 0.25ml plastic straws. XYGON™ cholesterol was incorporated into 5 straws which were cooled in the passive freezer as described in relation to Figure 2, configured to provide a -0.3°C/min cooling rate, before plunging into liquid nitrogen. The remaining 5 straws were cooled in a Planar R206 controlled rate freezer and seeded manually at -6°C.

The cooling profile for this machine was:

cool @ 5.0°C per min from 20 to -5°C
cool @ 0.2 -5 -6°C
seed during the second step
cool @ 0.5 °C per min from -6 to -32°C
plunge into liquid nitrogen

Embryos were thawed by immersion of the straws in water at 30°C, rinsed in several washes of culture medium with decreasing concentrations of cryoprotectant and incubated in culture medium overnight.

Of the five embryos frozen in the passive freezer, four were in excellent condition after culture and the fifth was still of an acceptable quality for transplanting. The embryos cooled in the Planar freezer were scored as (three) excellent and (two) still viable but not acceptable for transplanting.

Example 19 - Mammalian Cell Lines

A range of cultured mammalian cells were suspended in 91% FBS culture medium with 10% v/v DMSO, placed in 2.5ml plastic ampoules and then frozen in the passive freezer described above in relation to Figure 2b and configured to cool at 1.0°C per min. The cells were removed from the freezer when the samples had reached -18°C and were plunged directly into liquid nitrogen for a minimum storeage period of 24h.

Recovered cells were cultured in vitro and viable cell counts taken, based on the mean of two ampoules.

Cell Line	% Viability
TRK-49F Rat fibroblast	97
COS-7 Monkey kidney cells	98
3T3-Li Mouse fibroblast	95

CLAIMS

1. A method of freezing material comprising a liquid, the method comprising extracting heat from the material and varying the rate of heat extraction to compensate at least in part for latent heat being lost during freezing.

2. A method of freezing material comprising a liquid, the method comprising extracting heat from the material at a first rate while latent heat of fusion of the material is being lost from the material and the temperature of the material is not substantially falling and subsequently extracting heat from the material at a second rate when the temperature of the material falls, the first rate of heat extraction being greater than the second rate of heat extraction.

3. A method as claimed in claim 1 or 2, wherein the liquid is aqueous.

4. A method as claimed in claim 3, wherein latent heat removal is achieved in at most 50% of the time observed when following conventional blast freezing techniques at -30°C.

5. A method as claimed in any one of claims 1 to 4, wherein the material to be frozen comprises cells of biological origin.

6. A method as claimed in claim 5, wherein the cells are animal gametes or embryos.

7. A method as claimed in any one of claims 1 to 5, wherein the material to be frozen comprises a foodstuff.

8. A method as claimed in claim 8, wherein the foodstuff is for human consumption.

9. A method as claimed in claim 7 or 8, wherein the foodstuff comprises a vegetable, bread or another bakery product, meat, fish, sea food or fruit.

10. A method as claimed in claim 9, wherein the fruit is soft fruit.

11. A method as claimed in claim 7 or 8, wherein the foodstruff comprises ice cream and/or chocolate.

12. A method as claimed in any one of claims 1 to 11, which comprises initiating nucleation of solidifiable liquid.

13. A method as claimed in any one of claims 1 to 12, wherein the material being frozen is subjected to sound waves.

14. A method of freezing material comprising a liquid, the method comprising abstracting heat from the material and applying sound waves to the material by means of a non-liquid contact with the material.

15. A method of freezing material comprising a liquid, the method comprising abstracting heat from the material and applying sound waves to the material at a

power level of less than 2 W/cm^2.

16. A method of freezing material comprising a liquid, the method comprising abstracting heat from the material and intermittently applying sound waves to the material.

17. A method as claimed in any one of claims 13 to 16, wherein the sound waves are at a frequency of at least 16 kHz.

18. A method as claimed in any one of claims 13 to 17, wherein the sound waves are pulsed.

19. A method as claimed in any one of claims 13 to 18, wherein the sound waves are applied at a power level of less than 2 W/cm^2.

20. A method as claimed in claim 12, wherein nucleation is achieved at least partly by use of a chemical nucleator.

21. A method as claimed in any one of claims 1 to 20, wherein the material is being freeze-dried.

22. An apparatus for freezing material comprising a liquid, the apparatus comprising means for extracting heat from the material and control means for varying the rate of heat extraction to compensate at least in part for latent heat being lost during freezing.

23. An apparatus for freezing a material comprising a liquid, the apparatus comprising means for extracting

heat from the material at a first rate while latent heat of fusion of the material is being lost from the material and the temperature of the material is not substantially falling and means for subsequently extracting heat from the material at a second rate when the temperature of the material falls, the first rate of heat extraction being greater than the second rate of heat extraction.

24. A device for use in freezing material comprising a liquid, the device comprising a heat sink, insulating means at least partially surrounding the heat sink and means for holding, within the insulating means, material to be frozen, the device being adapted to withstand a temperature at which the material is frozen.

25. A device as claimed in claim 24, wherin the heat sink comprises metal.

26. A device as claimed in claim 24 or 25, wherein the insulating means comprises plastics material.

27. A method of freezing material comprising a liquid, the method comprising providing material to be frozen within insulating means, at least partially surrounding a cold heat sink with the insulating means, and providing a cold environment at least partially surrounding the insulating means.

28. An apparatus for freezing material comprising a liquid, the apparatus comprising means for abstracting heat from the liquid and means for applying sound waves

to the material, wherein (a) the sound waves are applied to the material by means of a non-liquid contact with the material and/or (b) the means for applying sound waves to the material is adapted to deliver the sound waves at a power level of less than 2 W/cm^2 and/or (c) the means for applying sound waves to the material is adapted to deliver the sound waves intermittently.

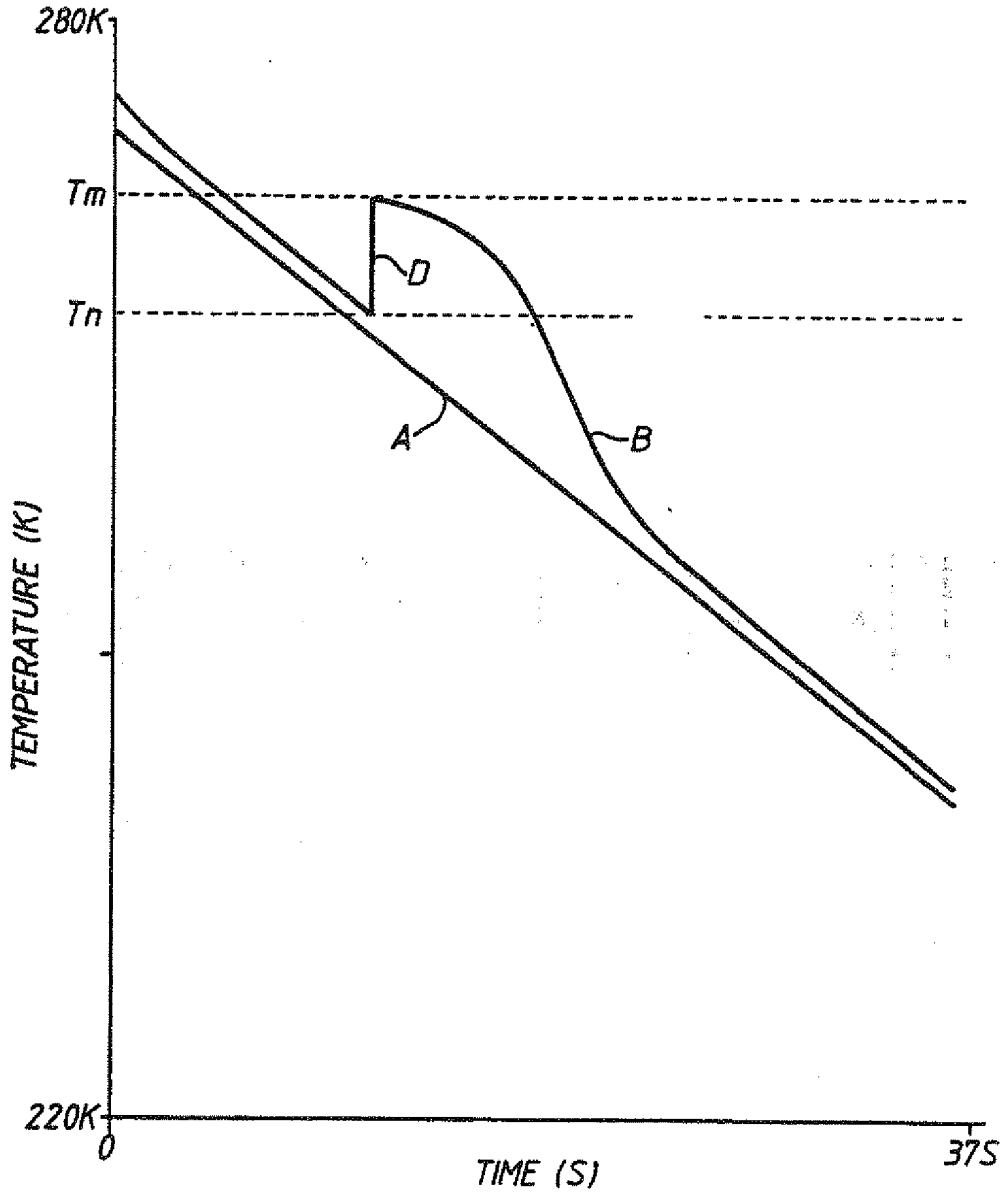


Fig.1.

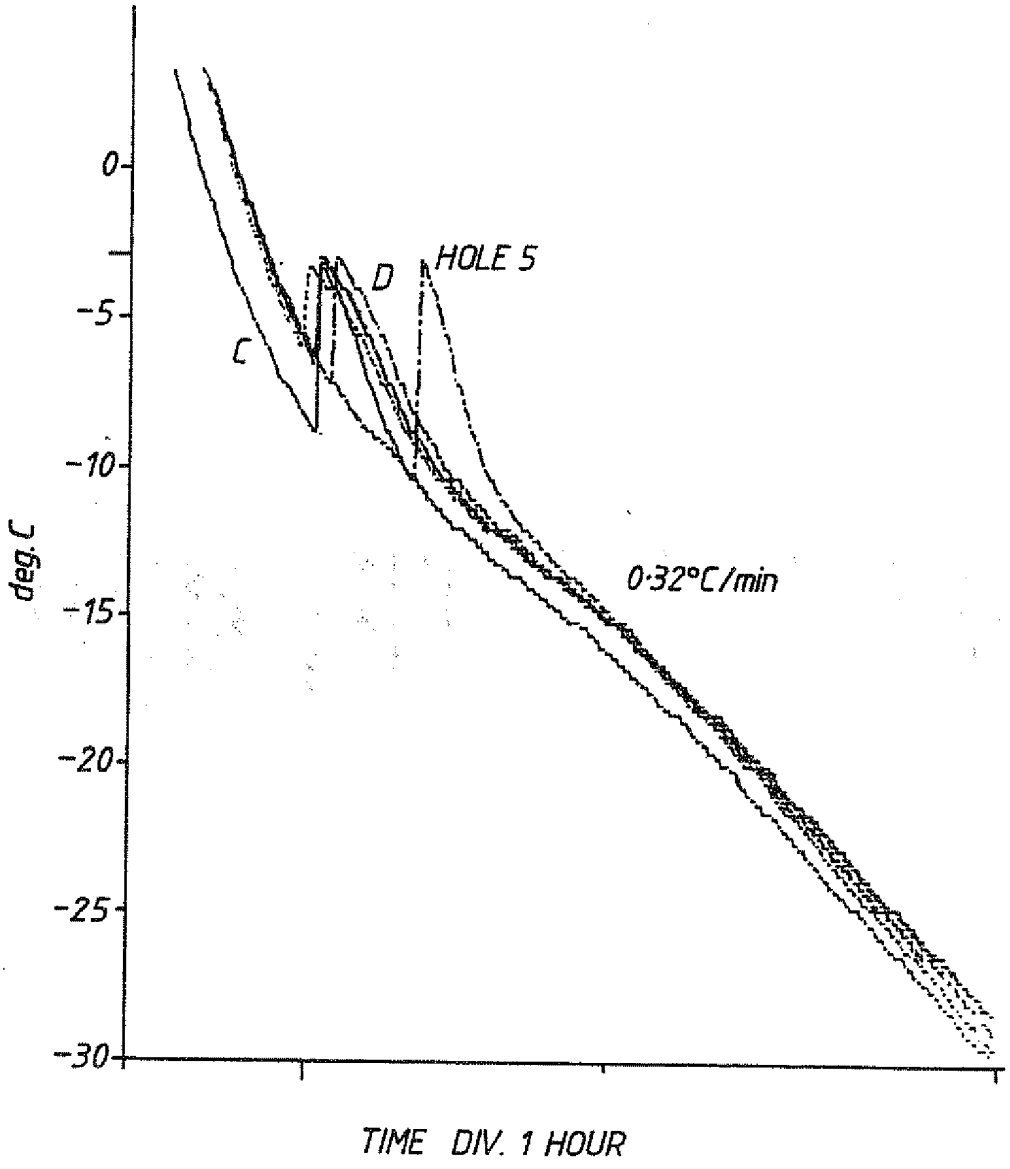
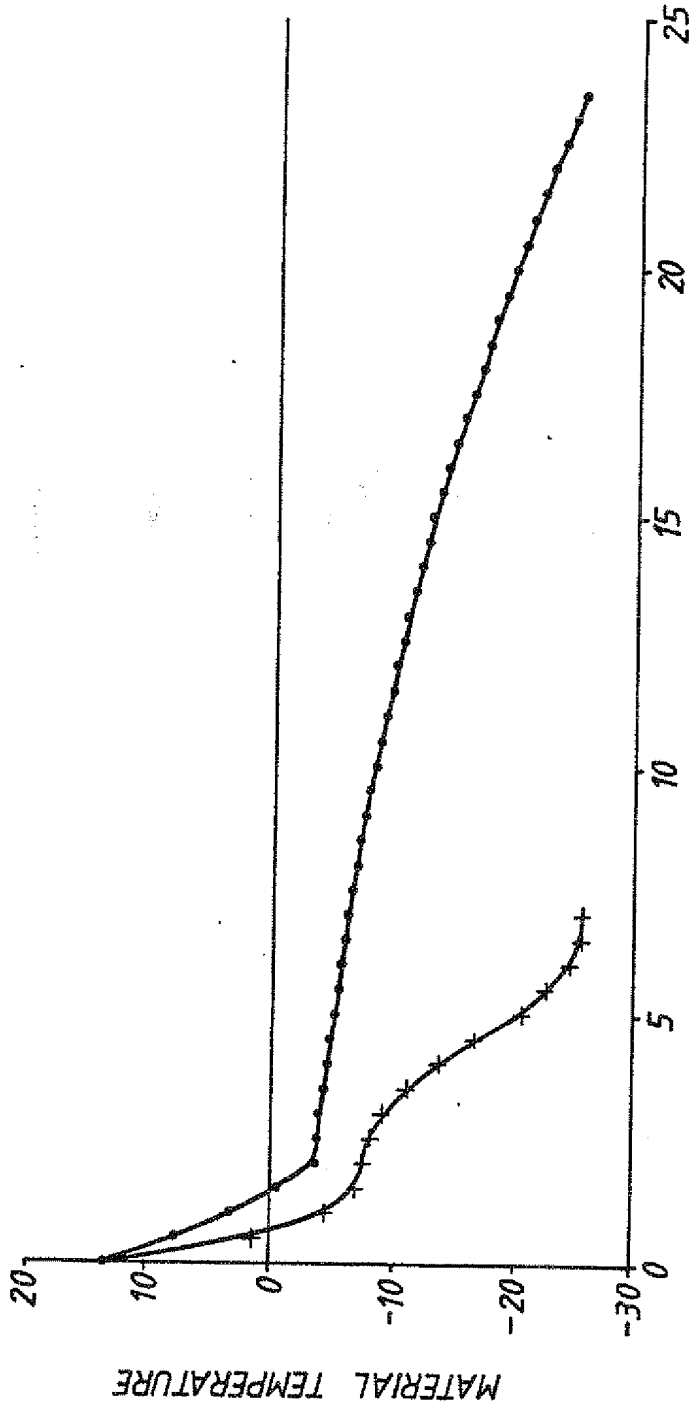


Fig. 3.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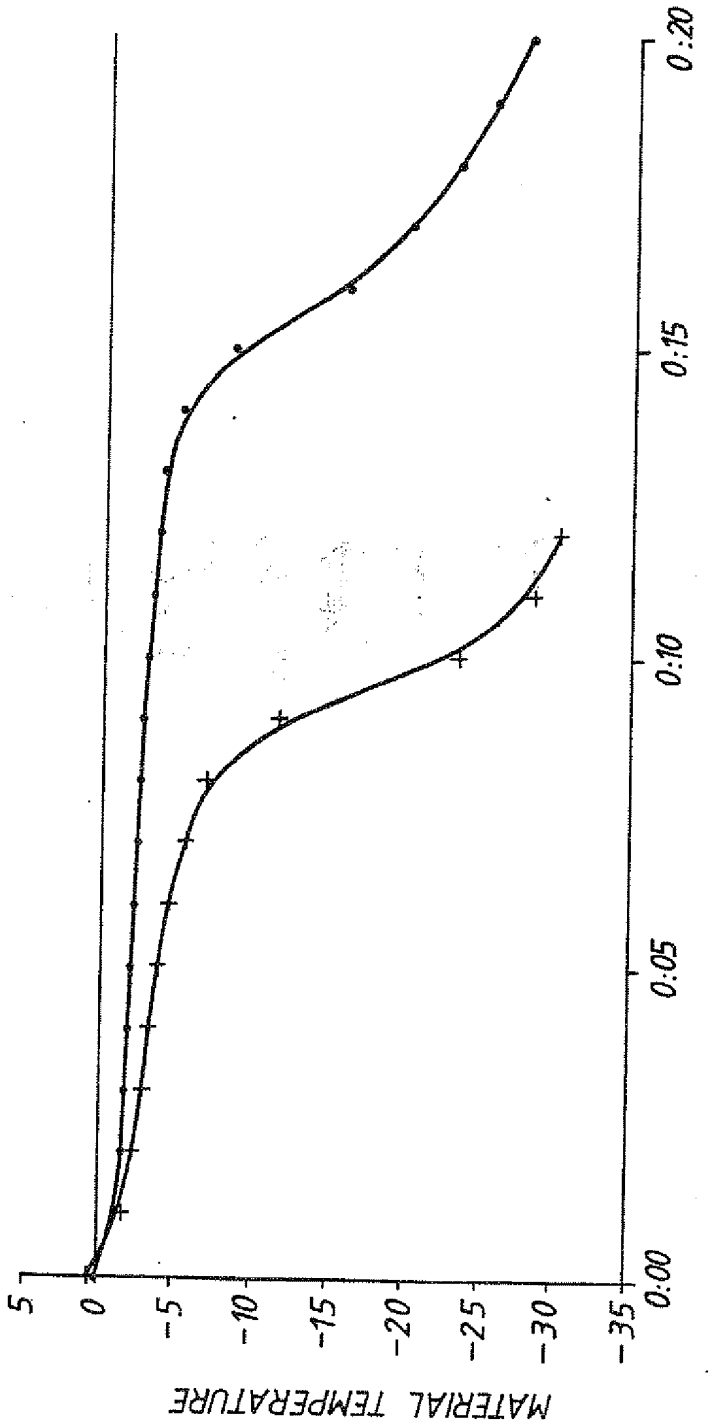
ELAPSED TIME (MINUTES)
BLAST FROZEN
PRESENT INVENTION

Fig. 4.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BLAST FROZEN
PRESENT INVENTION

Fig. 5.